The functional impact of CACNA1C and ANK3 risk genes for bipolar disorder on brain regional activation during emotional and cognitive tasks in healthy individuals, BD patients and their unaffected first-degree relatives

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The functional impact of CACNA1C and ANK3 risk genes for bipolar disorder on brain regional activation during emotional and cognitive tasks in healthy individuals, BD patients and their unaffected first-degree relatives

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Abstract

Bipolar Disorder (BD) is associated with increased familial risk and alterations in the function of large neural networks. There have been several studies that have examined the neural underpinnings of BD. Of particular relevance to this thesis are functional magnetic resonance imaging (fMRI) studies that have examined the neural correlates of affect and working memory processing in BD.

The first aim of my thesis was to examine the consistency and relative specificity of affect-related networks in BD. Using quantitative meta-analytic techniques I examined the neural correlates of facial affect processing in BD compared to healthy individuals and patients with Major Depressive Disorder (MDD) and schizophrenia (SZ). Emotional facial stimuli elicited increased activation in BD patients within the parahippocampus/amygdala, anterior cingulate cortex (ACC) and thalamus compared to all other groups. Decreased activation of the lateral ventral prefrontal cortex (VPFC) was found only when BD patients where compared to healthy individuals. Compared to BD patients, those with MDD showed greater activation in the dorsal ACC while those with SZ showed hyperactivation in posterior associative visual cortices.

The second aim of this thesis was to define the influence of key risk conferring single-nucleotide polymorphisms (SNPs) on the neural underpinnings of BD. To this purpose I focused on the CACNA1C (rs1006737) and ANK3 (rs10994336, rs9804190) which have the best supported genome-wide association evidence in BD. I analysed fMRI data from 41 BD patients, 25 unaffected first-degree relatives (RELs) and 46 healthy unrelated individuals (HI) while they were performing working memory (N-back) and facial emotion labelling tasks.

In the N-back task, HI carriers of the ANK3 rs10994336 risk-allele showed reduced activation in temporal regions while carriers of the ANK3 rs9804190-risk-allele showed inefficient overactivation in prefrontal regions. In BD patients and RELs, risk-alleles at either loci were associated with hyperactivation in the ventral ACC. Additionally, rs9804190 risk-allele carriers with BD evidenced hyperactivation within the posterior cingulate cortex.

In the facial emotion labelling task, for the ANK3 rs9804190 a significant group by genotype interaction was noted in the VPFC. The presence of the rs9804190 risk-allele was associated with reduced VPFC activation in BD patients and decreased activation in the RELs and HI. The ANK3 rs10994336 and CACNA1C rs1006737 risk-alleles were associated with increased activation in the inferior occipital and fusiform gyrus and the amygdala in all participants, regardless of group. A significant group by genotype interaction was again noted in the VPFC. The presence of the risk-alleles was associated with inefficient VPFC overactivation in HI and RELs but VPFC hypoactivation in BD patients.

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Distinct and original contributions

To date this work has generated 3 journal papers and 1 international conference presentation. Copies of the publications are provided in the Appendix. I worked closely with my supervisors, Prof. Frangou and Dr. Dima, to develop the full PhD proposal. A significant part of my thesis is based on the meta-analyses of publically available data sets. I was responsible for identifying the relevant literature, extracting and checking the data and conducting the meta-analyses. For chapter 5 and Chapter 6 the study population was drawn from the Vulnerability to Bipolar Disorders Study (VIBES) sample comprising subjects that had already been scanned and genotyped. I was responsible for conducting all analyses of data presented in this thesis as well as for the writing up of all the three published papers following guidance from Dr. Dima and Prof. Frangou. I wrote this thesis in its entirety under their supervision.

Details of the VIBES study including rationale, sample recruitment and investigational protocol have been published in**: Frangou S. Risk and resilience in bipolar disorder: rationale and design of the Vulnerability to Bipolar Disorders Study (VIBES). Biochem Soc Trans 2009; 37: 1085-1089.** A copy of the paper is appended in this thesis.

1. Bipolar Disorder

This Chapter provides a brief overview of Bipolar Disorder (BD). Specifically, it describes the clinical features of BD and presents estimates of the international prevalence of BD in the general population.

1.1 Introduction

Mood disorders include a broad range of psychopathological conditions whose clinical manifestation is characterized by affective disturbances. They are often associated with behavioural disturbances, cognitive impairments, thought disorders and other disruptions of sleep and appetite (American Psychiatric Association (APA), 2013). Moreover, it is important to point out that the increase in morbidity, mortality, and personal suffering associated with bipolar disorder is not simply a result of psychiatric symptoms. It has been shown that bipolar disorder is associated with a wide range of medical problems including cardiovascular disease, diabetes mellitus, obesity, and thyroid disease (Krishnan et al., 2005). Moreover, Kilbourne et al. (2004) reported that in BD patients the accumulation of key medical risk factors related to excessive nicotine use, use of alcohol and other drugs, and co-occurring anxiety disorders and eating disorders lead to the early onset of medical diseases with poor long-term outcomes. Taken together, these evidence suggested that the shorter lifespan observed in BD patients (from 13.5 to 32 years shorter) (Piatt et al., 2010) may be due to the combination of poor mental health and these general medical problems associated with the disease.

1.2 Clinical features of Bipolar Disorder

BD is associated with high levels of disability, morbidity, mortality and increased suicidal risk (Oswald et al., 2007). BD is defined by the presence of episodes of mania or hypomania and major depression. The APA (2013) defines a major depressive episode as lasting at least two weeks and including depressed mood or a loss of enjoyment in everyday activities. Manic episodes are defined by abnormal and persistently elevated mood that must last at least one week and may occur in conjunction with other symptoms including decreased need for sleep, hyperactivity, racing thoughts and an inflated self-esteem. A less intense manifestation of a manic episode that lasts less than four days is defined as being

hypomanic. It shows the same clinical symptoms but it is not severe enough to cause social or work impairment or to require hospitalization (Table 1-1).

The Diagnostic and Statistical Manual of Mental Disorders (DSM-5; APA, 2013) classifies BD in different categories that are not based on the underlying biology of the illness but rather on the consensus opinion of mental health professionals. The three major categories are:

• BD type I comprises patients who experience at least one manic episode. The age of onset is between 15 and 40 years, with a greater onset frequency in the early 20s (Morgan et al., 2005; Wittchen et al., 2003). In regard to the episode's polarity, women experience more depressive episodes, whereas manic and mixed episodes were equally frequent in both genders (Angst and Sellaro, 2000) (Table 1-2).

• BD type II includes patients who present at least one major depressive episode and at least one hypomanic episode. Comparative studies indicate that, compared to patients with BD type I, BD type II patients have a later age of onset (Merikangas et al., 2011), a higher number of relapses, a greater risk of recurrence (Vieta et al., 2008), lower rates of recovery, and a more chronic outcome characterized by more frequent depressive episodes with shorter euthymic periods (Angst et al., 1987; Bora et al., 2011) (Table 1-2).

• Cyclothymic Disorder is characterized by short periods of hypomania and short periods of mild depression. Cyclothymia refers to a milder form of BD, which manifestations do not reach levels that meet the diagnostic criteria for depression and hypomania (Table 1-2).

1.3 Prevalence of BD

Epidemiological studies have shown that BD is common in all cultures and ethnic groups (Weismann et al., 1996). A review of epidemiological studies in Europe suggests a prevalence of BD Type I of 1-2%, which rises to 6% when including bipolar spectrum disorders (Pini et al., 2005). Few studies have investigated the specific prevalence of BD type II; some suggest a greater prevalence of BD type II than BD type I (Vieta et al., 2008), others demonstrate the opposite (Faravelli et al., 1990; Sculli et al., 2004).

In the US population, Kessler et al. (1994) reported a rate of 1.6% in a representative US national sample of 8,098 participating in the National Comorbidity Survey (NCS). Several years later, first Kessler et al. (2005) and later Merikangas et al. (2007) replicated this result

in a sample of 9,282 US adults who participated in the National Comorbidity Survey Replication. These studies showed a lifetime prevalence rate for syndromal BD of 2.6% with a 12 month prevalence of 2.6% (Kessler et al., 2005) and 1.4% (Merikangas et al., 2007). Lifetime prevalence of BD increased to 4.5% when considering sub-threshold expressions (Merikangas et al., 2007).

Finally, the World Mental Health Survey Initiative, involving 61,392 adults in eleven countries in North and South America, Europe, and Asia, reported lifetime prevalence estimates of syndromal BD of 1.4 % (Merikangas et al., 2011).

1.4 Comparison between the two major classification systems for Bipolar Disorder: the International Classification of Diseases (ICD-10) and the Diagnostic and Statistical manual of Mental Disorders (DSM-5)

The prevailing diagnostic systems are the 10th revision of the International Classification of Diseases, ICD-10 (World Health Organization, 1993) and the 5th edition of the Diagnostic and Statistical Manual for Mental Disorders (DSM-5) (American Psychiatric Association, 2013). These two systems operate with diverging definitions of bipolar disorder. For the diagnosis of Bipolar type I, the DSM-5 requires at least one full-blown episode of mania and at least both a major depressive and a hypomanic episode for the diagnosis of Bipolar type II. On the other hand, the ICD-10 defines bipolar disorder by at least two episodes of altered mood state and activity level. Consequently, the ICD-10 system does not differentiate between bipolar type I and bipolar type II, and as long as there has been at least two hypomanic episodes, no major depressive episode is required for a diagnosis of bipolar disorder. Thus, the ICD-10 system can be said to define bipolar disorder in a broader manner than the DSM5, and patients with the same symptomatic picture can fall within different diagnostic groups, depending on the system being used (see Table 1-3 with the ICD-10 criteria for Bipolar Disorder).

1.5 Economical and Societal Burden

People living with BD suffer substantial functional impairment and as one of the top 10 leading causes of disability worldwide its burden outranks all cancers and primary neurologic disorders (e.g. epilepsy and Alzheimer's disease; Merikangas et al., 2011), with sufferers estimated to be unable to maintain proper work role function over 30% of the time (Judd et al., 2008). In the UK the direct (e.g. the unit resource costs) and indirect costs

(e.g. excess unemployment, absenteeism and suicide) attributable to BD are almost 2 billion pounds per year (Das Gupta et al., 2002). These costs remain high as in 2009/2010 the annual cost of BD to the National Health Service (NHS) reached £342 million (Young et al., 2011). Estimates of the total costs of BD in the USA may be even higher exceeding 45.2 billion dollars (Wyatt & Henter, 1995). Emerging evidence also demonstrated the association between BD and psychosocial functioning with profound disruptions in work productivity and social functioning, including high unemployment rates and relationship breakdowns (Kessler et al., 2006; Ruggero et al., 2007; Zimmerman et al., 2010). Specifically, several studies reported impairments in psychosocial functioning in BD patients with reduced occupational, interpersonal and psychological adjustment associated not only with acute phases of the illness but even in fully remitted patients (Judd et al., 2008; Martinez-Aran et al., 2007; Simonsen et al., 2010). One study, which followed-up 128 patients with BD six months after admission for a manic episode, showed that 77% of patients achieved syndromal recovery whereas only 29% achieved functional recovery. Considering only those patients who achieved syndromal recovery, almost 65% failed to achieve functional recovery (Tohen et al., 2000). However, a study by Judd et al. (2008) also reported that the severity of psychosocial dysfunctions was associated with the level of affective symptom severity, with depressive symptoms considered more disabling than hypomanic ones and as disabling as manic symptoms.

Table 1-1 DSM-5 criteria for the manic, hypomanic and major depressive episodes

MANIC EPISODE		**HYPOMANIC EPISODE**	**MAJOR DEPRESSIVE EPISODE**
SymptomDuration	Episode must last at least 1 week, with symptoms present nearly every day	Episode must last at least 4 consecutive days, with symptoms present nearly every day	Episode must last at least 2 weeks, with symptoms present nearly every day
Core Symptoms	Persistently elevated mood, expansive, or irritable mood and abnormally and persistently increased goal directed activity or energy	Abnormally and persistently elevated, expansive, or irritable mood and abnormally and persistently increased activity or energy	Depressed mood Loss of interest or pleasure
Auxiliary Symptoms	- Inflated self-esteem or grandiosity - Decreased need for sleep - More talkative than usual or pressure to keep talking - Flight of ideas or subjective experience that thoughts are racing - Distractibility - Increase in goal-directed or psychomotor agitation - Excessive involvement in activities that have a high potential for painful consequences	- Inflated self-esteem or grandiosity - Decreased need for sleep - More talkative than usual or pressure to keep talking - Flight of ideas or subjective experience that thoughts are racing - Distractibility - Increase in goal-directed or psychomotor agitation - Excessive involvement in activities that have a high potential for painful consequences	- Weight/appetite loss/gain - Insomnia/hypersomnia - Psychomotor agitation or retardation - Fatigue/loss of energy - Feelings of worthlessness - Diminished ability to think/ Concentrate - Recurrent suicidal thoughts
Inclusion Criteria	Core symptoms + 3 or more auxiliary Symptoms PLUS Significant distress OR Social Impairment	Core symptoms + 3 or more auxiliary Symptoms	At least one core Symptom + 4 or more auxiliary Symptoms PLUS Significant distress OR Social Impairment
Exclusion Criteria	Not substance related (e.g., a drug of abuse, a medication, other treatment) Not associated with any other medical condition	Not substance related (e.g., a drug of abuse, a medication, other treatment) Not associated with any other medical condition	Not substance related (e.g., a drug of abuse, a medication, other treatment) Not associated with any other medical condition

Table 1-2 DSM-5 classification of Bipolar Disorder

Bipolar I Disorder
A. Criteria have been met for at least one manic episode (see Table 1-1). B. The occurrence of the manic and major depressive episode(s) is not better explained by schizoaffective disorder, schizophrenia, schizophreniform disorder, delusional disorder, or other specified or unspecified schizophrenia spectrum and other psychotic disorder.
Bipolar II Disorder
A. Criteria have been met for at least one hypomanic episode (see Table 1-1) and at least one major depressive episode (see Table 1-1). B. There has never been a manic episode. C. The occurrence of the hypomanic episode(s) and major depressive episode(s) is not better explained by schizoaffective disorder, schizophrenia, schizophreniform disorder, delusional disorder, or other specified or unspecified schizophrenia spectrum and other psychotic disorder. D. The symptoms of depression or the unpredictability caused by frequent alternation between periods of depression and hypomania causes clinically significant distress or impairment in social, occupational, or other important areas of functioning.
Cyclothymic Disorder
A. For at least 2 years (at least 1 year in children and adolescents) there have been numerous periods with hypomanic symptoms that do not meet criteria for a hypomanic episode and numerous periods with depressive symptoms that do not meet criteria for a major depressive episode. B. During the above 2-year period (1 year in children and adolescents), the hypomanic and depressive periods have been present for at least half the time and the individual has not been without the symptoms for more than 2 months at a time. C. Criteria for a major depressive, manic, or hypomanic episode have never been met. D. The symptoms in Criterion A are not better explained by schizoaffective disorder, schizophrenia, schizophreniform disorder, delusional disorder, or other specified or unspecified schizophrenia spectrum and other psychotic disorder. E. The symptoms are not attributable to the physiological effects of a substance (e.g., a drug of abuse, a medication) or another medical condition (e.g., hyperthyroidism). F. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.

Table 1-3 The ICD-10 Criteria for Bipolar Disorder

F31	Bipolar affective disorder is a disorder characterized by two or more episodes in which the patient's mood and activity levels are significantly disturbed, this disturbance consisting on some occasions of an elevation of mood and increased energy and activity (hypomania or mania) and on others o a lowering of mood and decreased energy and activity (depression). Repeated episodes of hypomania or mania only are classified as bipolar. Includes: manic-depressive illness, psychosis or reaction. Excludes: bipolar disorder, single manic episode and cyclothymia

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2. Emotion and Cognition in Bipolar Disorder: Evidence from Neuroimaging and Neuropsychological findings

2.1 Structural Brain changes in BD

Neuroanatomical studies have illustrated significant differences between BD patients and healthy individuals in several cortical and subcortical regions. Among the most consistently reported abnormalities are the ventricular enlargement and the presence of white matter hyperintensities (Kempton et al., 2008; Strakowski et al., 2002). Lateral ventricles were found to be increased in BD patients and notably this feature seemed to correlate with the number of manic episodes (Strakowski et al., 2002). The lateral ventricular enlargement has also been reported by an International collaborative mega-analysis of published and unpublished data on 321 BD patients and 442 healthy individuals. This study also reported that BD patients compared to healthy individuals showed increased temporal lobe (mean volume estimated between 2.1 and 12.6 mm larger) and putamen volumes (Hallahan et al., 2011). In addition to these findings, gray matter (GM) abnormalities have been observed in the prefrontal cortex, with GM reductions in BD patients compared to healthy individuals (Strakowski et al., 2005). The study by Lopez-Larson et al. (2002) found that BD patients showed smaller GM volumes in the left superior, middle and right prefrontal regions and suggested that the longer duration of affective illness was associated with smaller left inferior prefrontal GM volumes. Similarly, reductions in GM volumes was also found by Brambilla et al. (2002) in the left dorsolateral prefrontal cortex (DLPFC) in unmedicated BD patients compared to healthy individuals.

Additionally, a recent review reported GM volumes reductions in the subgenual ACC in BD patients who were in remitted or euthymic states of the illness which persisted even during antidepressant treatment (Drevets et al., 2008). GM volumes reduction in the ACC was also suggested by a meta-analysis by Bora et al. (2010) together with the evidence of decreased GM volumes in the fronto-insular cortex.

Anatomical studies investigating GM volumes in subcortical regions have not yielded consistent findings. A review by Strakowski et al. (2005) reported both increased and decreased GM volumes in BD patients in the amygdala, thalamus and the striatum. A meta-

analysis by Ng et al. (2009) reported no significant GM changes in the thalamus and amygdala-hippocampal regions, with few studies associated with either increases or decreases in these structures. These inconsistent evidence may be due to variations in age and in lithium use across studies (Usher et al., 2010a, Usher et al., 2010b). It has been widely shown that lithium has neurotrophic effects (Manji et al., 1999) and it has been supported by several findings that showed the association of lithium with increased GM volumes in BD patients (Bearden et al., 2007; van Erp et al., 2012). The mega-analysis by Hallahan et al. (2011) further supported these evidence by reporting increased hippocampal and amygdala volumes in BD patients taking lithium compared with BD patients not treated with lithium and healthy individuals. Finally, an international collaboration for the study of BD (ENIGMA consortium-bipolar group) performed one of the largest studies exploring the subcortical volumes in a sample of 1,745 BD patients and 2,613 healthy individuals. The authors reported that BD patients showed significantly lower volumes in the hippocampus, thalamusand amygdalaas well as larger lateral ventricles compared to healthy individuals (Hibar et al., under revision).

2.2 Functional MRI

FMRI provides an indirect measure of neuronal activity by measuring changes in blood-oxygen-level dependent (BOLD) signal. Over the last two decades, fMRI studies have shown functional alterations in specific brain areas that are closely related to BD. This chapter provides a critical appraisal of the findings to date in patients with BD while performing affective and non-affective tasks.

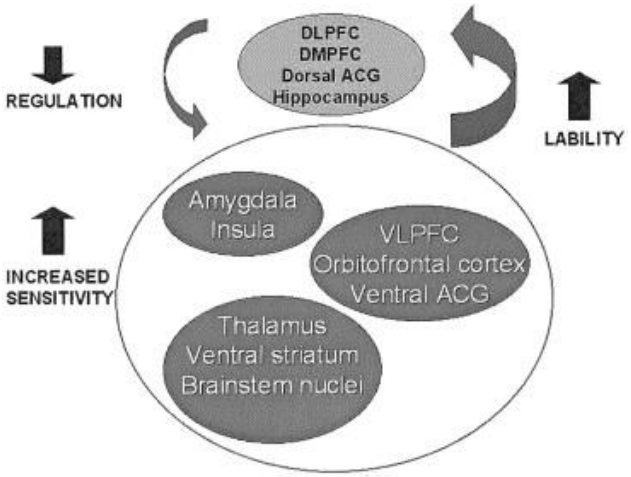
2.2.1 Affective cognition

The affective processing network

From a neurobiological perspective, it has been proposed that the normal perception of the emotions is performed by two neural circuits (Phillips et al., 2003). A *ventral system* that includes the hypothalamus, the insula, the amygdala, the ventral striatum, the ventral anterior cingulate cortex (ACC) and the prefrontal cortex, and *a dorsal system* that is composed by the hippocampus, the dorsal ACC and the prefrontal cortex. According to this model, the ventral system is responsible for the evaluation of the emotional stimuli as well as the regulation of neuro-vegetative responses. The dorsal system, however, is involved in

the cognitive aspect of the emotion and in the regulation of the affective state. Between these two systems there is a reciprocal functional relationship due to their reciprocal anatomical connections (Fig. 2-1).

Figure 2-1 Schematic model of the dorsal and ventral systems underpinning the perception and regulation of the emotions (Phillips et al., 2003).

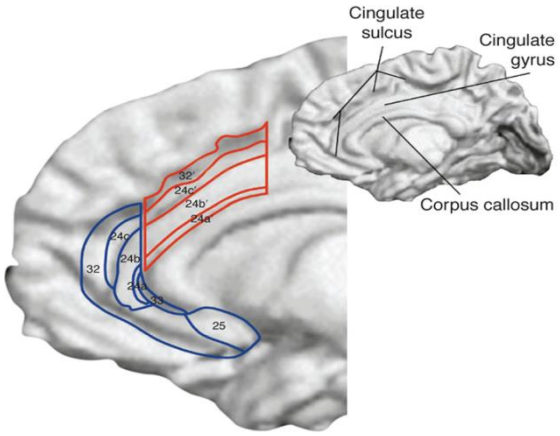


Although the ventral and dorsal systems are essential for normal emotional response, there are key regions within these systems that deserve more attention. Specifically, the amygdala is the core region of the limbic system. It integrates external and internal information and has direct output connections to nuclei that control physiologic, autonomic and behavioural fear-induced responses (Duvarci et al., 2014). It also has extensive connections with the prefrontal cortex and thus to neocortical circuits involved in cognitive processes which integrate the emotional content of the stimuli (Ghashghaei et al., 2007). A recent voxel-based meta-analysis by Fusar-Poli et al. (2009) generated detailed neurofunctional maps of emotional facial processing in an overall sample of 1,600 healthy individuals. Specifically for the amygdala, the authors reported increased activation of the amygdala in response to the presentation of facial expressions of fearful, happy and disgusted faces.

The ACC is extremely diverse and complex and can be divided into two components based on its functional organization: the ventral ACC and the dorsal ACC (Bush et al., 2000). The ventral ACC is associated with emotional behaviour and includes the subgenual ACC

(Brodmann area [BA] 25), the pregenual BA 33 and the rostral ACC (BA 24) (Devinsky et al., 1995). Further, the ventral ACC is widely identified as connected to several subcortical regions including the amygdala, the thalamus and the ventral striatum and is associated with the regulation of the emotional behaviour in response to emotional stimuli (Paus et al., 2001). On the other hand, the dorsal ACC (dorsal BA 24 and BA 32) is connected with the prefrontal and parietal cortex and it is involved in emotional appraisal and cognitive conflict (Bush et al., 2000) (Fig. 2-2).

Figure 2-2 Representation of the two components of the ACC: the cognitive dorsal ACC (red) and the emotional ventral ACC (blue) (Bush et al., 2000).



Finally, the prefrontal cortex with its extensive connections with motor, perceptual and limbic regions of the brain is considered a key region for the emotional processing, especially the DLPFC and orbito-frontal cortex (OFC). Positron emission tomography (PET) studies have shown that the DLPFC (BA 9, BA 46) was associated with increased activation in several experimental conditions, including sad mood (Pardo et al., 1993) and guilt (Shin et al., 2000) induction, and recall of emotional material (Reiman et al., 1997). By contrast, the OFC (BA 11, BA 47) has direct connections with the amygdala and appears to play a crucial role in representation of the reward value of a stimulus and therefore in goal-directed behaviours (Schoenbaum et al., 2011).

The clinical features of BD, including mood lability and irritability, are suggestive of possible dysfunction in affective processing and therefore of a dysregulation of the brain regions

described above. The majority of neuropsychological and fMRI studies that investigated the emotional processing in BD patients used facial affect recognition tasks. This is because emotional facial expressions are an important part of social communication and play a crucial role in interpersonal interaction. Evidence form fMRI studies investigating the neural organization of facial emotion processing in healthy individuals suggested the involvement of the ventral and dorsal systems as well as occipital temporal regions (e.g, the fusiform gyrus and superior temporal gyrus) (Fusar-Poli et al., 2009; Vytal and Harmann, 2010), areas implicated in perception and recognition of face identity and emotional expressions (Adolphs et al., 2002). In these tasks, the participant is shown a picture of someone showing an emotional expression and the participant is asked to identify which emotion they think the person is expressing. These tasks may vary in the selection of the emotions, the stimuli used and the task format that involved either the implicit or the explicit recognition of a facial emotion. While the explicit tasks involve labelling the emotions, judging the intensity of the emotions or matching emotions, the implicit tasks refer to the judgment of other facial cues (e.g. gender).

In the following sections I describe the main neuropsychological deficits and neuronal dysfunctions in BD patients observed while processing the aforementioned facial affect recognition tasks.

Neuropsychological studies in BD

Neuropsychological findings have suggested altered facial affect processing in BD patients in either depressed, manic or euthymic state compared to healthy individuals (Wessa et al., 2009). Gray et al. (2006) investigated the sensitivity to the facial expressions of six basic emotions in a sample of 14 depressed BD patients and 9 manic BD patients. The authors found a significantly reduced sensitivity to happy facial expressions and a general negative effect in depressed but not in manic patients.Moreover, the study by Schaefer et al. (2010) reported deficits in the emotional expression multimorph task, characterized by facial emotions displayed in gradations from neutral to 100% emotional expression of happy, sad, surprised, fearful, angry, and disgusted. The authors showed that depressed BD patients exhibited general decrease in sensitivity to facial expressions compared to healthy

individuals and required more intense facial expressions before they correctly identified the emotion.

Getz et al. (2003) found that manic BD patients showed deficits in the facial affect labelling task where they made more errors than healthy individuals. In addition, Lembke et al. (2002) investigated the performance in the facial affect recognition task of manic BD and euthymic patients with BD-I and BD-II diagnosis. The authors reported that manic BD patients showed the greatest impairments overall in the facial affect recognition task with worse recognition of fear and disgust compared to healthy individuals whereas euthymic BD-II patients showed enhanced fear recognition compared to manic and euthymic BD-I patients. Moreover, Bozikas et al. (2006) explored the performance of euthymic BD patients in tests assessing identity (Kinney's Identity Matching Test) and matching facial emotion expressions (Kinney's Affect Matching Test). The authors found impairments in the affect matching task without, though, providing details on each of the different emotions. Martino et al. (2008) found that euthymic BD patients showed impairments in the recognition of fear and disgust compared to healthy individuals while processing a facial emotion recognition task. Hoertnagl et al. (2011) examined the facial emotion recognition abilities by using the facially expressed emotion labelling test in 47 euthymic BD patients. The authors showed that BD patients were significantly impaired in the recognition of happiness and disgust compared to healthy individuals. Finally, in a similar task, David et al. (2014) explored the performance of 110 BD patients in different mood states (depressive, manic and euthymic) during a facial emotion recognition test (FER) that included faces depicting happiness, sadness, surprise, disgust, anger and fear. The authors showed that BD patients has lower overall FER performance as well as lower FER scores in fear, happiness and surprise tests when compared to healthy individuals.

FMRI studies in BD

To date, several FMRI studies have investigated the processing of emotional information in BD patients (Townsend et al., 2012; Strakowski et al., 2012; Chen et al., 2011). Although the facial emotion recognition task is the most extensively used paradigm for exploring emotional processing in BD patients, this section also briefly describes the major findings related to the neural activation in other emotional tasks, including the affective go/no-go,

the emotional Stroop and the Iowa Gambling tasks. These tasks aim to explore the impact of emotional factors on cognitive processes in order to investigate how emotions modulate brain activity in regions that subserve cognitive functions (Drevets et al., 1998).

The Affective Go/no-go Task

The affective Go/no-go task involves three conditions (positive, negative and neutral) and participants are instructed to indicate when a positive valenced stimuli (words or faces) appeared in the context of negatively valenced distracters (Jongen et al., 2007). It has been reported that this task engages regions within the prefrontal cortex and ACC (Shafritz et al., 2006; Elliot et al., 2000).

Neuropsychological studies reported that BD patients showed deficits in performing this task (Murphy et al., 1999; Rubinzstein et al., 2000). Murphy et al. (1999) explored the performance of manic and depressed BD patients by employing the Cambridge Neuropsychological Test Automated Battery (CANTAB) which also included an affective Go/no-go task. In this task, the authors reported that manic BD patients showed mood-congruent biases for sad and happy targets; patients were slower when responding to the presentation of sad word compared to happy word targets and made more omission errors, with more happy missed targets than sad targets. On the other hand, depressed BD patients were slower when responding to the presentation of happy word as compared to sad word targets. In addition, Rubinzstein et al. (2000) suggested a tendency towards significance in response latency in the same affective Go/no-go task employed by Murphy et al. (1999), with slower reaction time in euthymic BD patients compared to healthy individuals.

From a neurobiological point of view, three fMRI studies explored the neural activation associated with this task during euthymic (Wessa et al., 2007; Hummer et al., 2013), depressed (Hummer et al., 2013) and manic (Hummer et al., 2013; Elliot et al., 2004) states. However, while Wessa et al. (2007) and Hummer et al. (2013) used an affective Go/no-go task with emotional faces as stimuli, Elliot et al. (2004) employed an affective Go/no-go task with emotional words. These studies found no differences in reaction time and accuracy measures in the diagnostic group employed. Wessa et al. (2007) showed that euthymic BD patients exhibited increased activity in the temporal and the orbital cortex, the insula, the

caudate, the dorsal anterior and posterior ACC when inhibiting emotional stimuli compared to healthy individuals. In addition, Hummer et al. (2013) reported that euthymic BD patients showed increased insula and inferior frontal activation during happy and sad face inhibition respectively compared to healthy individuals. For manic and depressed BD patients, the authors also showed increased activation in the putamen, insula and prefrontal cortex while inhibiting sad faces compared to healthy individuals. In addition, increased putamen and insula activation was also found in manic BD patients while inhibiting neutral stimuli. Finally, Elliot et al. (2004) reported that manic BD patients compared to healthy individuals showed attenuated middle frontal gyrus response and increased activation in the inferior temporal and frontal gyrus, middle frontal gyrus and superior frontal polar region when all the emotional conditions were compared with a neutral condition. Manic patients showed also enhanced response to emotional relative to neutral distracters in the ventrolateral prefrontal cortex (VLPFC) and DLPFC.

The Emotional Stroop (eStroop) Task

In the eStroop task, participants are instructed to pay attention to the colour of the words and to inhibit the emotional content of the word presented. Slower responses are indicative of the interference effect of the words' content on the information processing abilities (Compton et al., 2003). Regions that have been consistently implicated in this task are the ACC (Whalen et al., 1998), the amygdala and the prefrontal cortex (Compton et al., 2003).

Two fMRI studies explored the neural activation in euthymic BD patients while performing the eStroop task, and specifically while comparing emotionally valent and neutral words (Lagopoulos et al., 2007; Malhi et al., 2005). Both studies showed that euthymic BD patients have decreased activation in the VLPFC compared to healthy individuals. However, the two studies found different pattern of activation in subcortical regions. Malhi et al. (2005) reported decreased activation of the amygdala, thalamus and putamen whereas Lagopoulos et al. (2007) found significantly greater activity in the hippocampus and amygdala in euthymic BD patients compared to healthy individuals.

In summary, the fMRI studies that employed the affective Go/no-go and the eStroop tasks provided evidence of deficits in response inhibition in BD patients, and particularly in the

emotional modulation of this cognitive process. With regard of neural activation, these studies demonstrated a cognitive-emotional interference in BD patients indicated by altered prefrontal as well as subcortical activation, although the direction of these alterations is still not clear.

The Iowa Gambling Task (IGT)

The IGT is an affective decision making task which is composed by four decks of cards (A,B,C,D) that have to be chosen by the participant. Behind each deck there is a positive or negative monetary reward; decks A and B contain high risk cards as the reward and penalty money is higher, which results in net loss whereas deck C and D have lower gains but also lower penalties making them advantageous overall. The aim of this task is to make as much money as possible. It has been shown that the neural systems involved in reward processing engage the amygdala, striatum, thalamus and the prefrontal cortex, including OFC and ACC (Elliot et al., 2003).

Neuropsychological studies suggested that manic BD patients underperformed in this task (Clark et al., 2001) and similar decision making tasks (Cambridge Gamble Task) (Murphy et al., 2001) as did unmedicated depressed BD-II patients compared to healthy individuals(Taylor Tavares et al., 2007).

Two fMRI studies explored the neural activity in euthymic BD patients compared to healthy individuals while processing of the IGT (Frangou et al, 2008; Jogia et al., 2012). Jogia et al. (2012) suggested that euthymic BD patients showed increased activation in the ventro-medial frontopolar prefrontal cortex as well greater activation of the ACC and the posterior cingulate cortex. No differences in reaction time and performance were found in this study. Frangou et al (2008) also reported a significant difference in neural responses but not in task performance. In this study, the authors found that euthymic BD patients showed attenuated neural activation throughout the prefrontal cortex and increased activation in lateral and polar temporal regions. Finally, a PET study by Rubinsztein et al. (2001) found that manic BD patients showed increased activation in the ACC and reduced activation in the frontopolar cortex and VLPFC compared to healthy individuals. The authors reported no group differences in performance.

In summary, these studies suggest that while processing of an affective decision making task, BD patients showed altered activation of region within the prefrontal cortex, including the VLPFC and the frontopolar cortex, as well as increased activation in the limbic regions, such as the ACC.

The Facial Emotion Recognition Task

As mentioned in the previous paragraphs, the facial emotion recognition task has been employed by the majority of fMRI studies to investigate emotional processing in BD patients in different affective states compared to healthy individuals (Townsend et al., 2012). It has been reported that this task engages several brain regions linked to limbic, visual, temporoparietal and prefrontal areas (Fusar-poli et al., 2009).

Specifically for manic BD patients, Lennox et al. (2004) showed decreased amygdala and ventral ACC and increased posterior cingulate and insula activation in response to sad but not happy facial expressions. Chen et al. (2006) suggested increased activation in the middle temporal gyrus, parahippocampal gyrus and thalamus to fearful faces and the fusiform gyrus to sad faces. For happy faces, the authors reported increased activation in left superior frontal gyrus, ventral prefrontal cortex and middle temporal gyrus.

For depressed BD patients, Chen et al. (2006) reported overactivity in fronto-striato thalamic systems in response to happy faces whereas Altschuler et al. (2008) suggested reduced activations in the VLPFC and DLPFC as well as increased activation in the middle prefrontal cortex in response to faces with negative emotions. Almeida et al. (2010) reported increased amygdala activation in response to mildly sad emotional faces. Finally, Lawrence e al. (2004) explored neural responses to mild and intense expression of fear, happiness, and sadness in euthymic and depressed BD patients. The authors found a positive correlation with the degree of depression and increased activation in the hippocampus while viewing sad facial affects.

For euthymic BD patients, the study by Hassel et al. (2008) reported decreased activation in the DLPFC in response to happy and fearful faces. Malhi et al. (2007) found decreased middle and inferior frontal gyrus, precuneus and insula as well as increased middle occipital gyrus in response to disgust and increased superior temporal gyrus and inferior parietal

gyrus in response to fearful faces. Surguladze et al. (2010) suggested increased medial prefrontal cortex activation in response to both fearful and happy faces as well as increased activation of the amygdala in response to happy faces. Moreover, two studies also found hyperactivation in striatal regions, including the caudate and putamen (Surguladze et al., 2010; Hassel et al., 2008).

In summary, these results suggested an increased activation of prefrontal regions and decreased activation of limbic regions, including the amygdala, in BD patients compared to healthy individuals. However, the valence of the emotions as well as the different mood states may vary this pattern of activation.

2.2.2 Non affective cognition

Over the last decade there has been increased interest in executive dysfunctions associated with BD. In this paragraph, I review the main findings with regard to the neuropsychological and fMRI dysfunctions that affect BD during the processing of a broad range of cognitively demanding tasks.

The neural brain network underpinning executive functions

Executive functions are of higher order cognitive abilities. They allow humans to organize and implement complex actions to achieve an adaptive behaviour. In humans, executive functions include: planning, judgement, abstract thinking, initiation and inhibition of behaviour, anticipation of outcomes, and the adaptation of behaviour to changing environmental situations (Alvarez et al., 2006). It is widely agreed that the neural substrate of the executive functions are frontal circuits that if damaged can lead to a "dysexecutive syndrome" (Duffy and Campbell, 1994). However, fMRI studies have also suggested the involvement of other areas activated during the performance of executive functional tasks including parietal and temporal regions (Szameitat et al., 2002).

Although fMRI studies reported the presence of several neural networks across the brain (Power et al., 2011), the fronto-parietal network is one of the most recognized for the top-down regulation of lower order processes and therefore on cognitive control operations (Zanto et al., 2013). The main regions that are part of this network and linked to executive

functions are: the DLPFC, VLPFC, the dorsal ACC, the OFC and the parietal cortex. These regions are variously responsible for response inhibition, working memory, verbal fluency, signalling motivational salience, selective attention, error detection and conflict monitoring (Behrmann et al., 2004; Braver et al., 2001; Bush et al., 2000; MacDonald et al., 2000; Stuss et al., 2000).

Neuropsychological studies in BD

The presence of cognitive deficits during the acute phases of the disease is widely recognized in the literature (Latalova et al., 2011; Martinez-Aran et al., 2004; Quraishi and Frangou 2002). However, several meta-analyses demonstrated that neurocognitive deficits persist even during the euthymic phase (Arts et al, 2008; Bora et al, 2009; Mann-Wrobel et al., 2011; Robinson et al, 2006; Torres et al., 2007) and therefore contribute to the absence of a complete functional inter episode recovery with significant psychosocial difficulties also present during periods of euthymia (Martino et al., 2009).

These studies obtained overlapping results, identifying worse performance (medium-large effect sizes) of euthymic BD patients in executive functions (working memory, verbal fluency), memory, sustained attention and verbal learning. The most recent meta-analysis by Mann-Wrobel et al. (2011) showed, in a sample of 1,026 euthymic BD patients and 1,384 healthy individuals, that there is a generalized neurocognitive impairment with medium-large effect sizes in BD patients compared to healthy individuals. Specifically, the authors explored several cognitive measures that can be grouped in seven cognitive domains, including speed of processing, episodic memory, executive functioning, working memory, fluency, perceptual\problem-solving, and intellectual\verbal ability. They also reported the effect of possible confounding variables including sex, education, age and illness duration. Sex did not influence the neurpsychological functioning whereas higher education, age and illness duration were correlated with less neurocognitive impairment. Finally, a longitudinal study by Chaves et al. (2011) explored the performance on a neuropsychological test battery that covers a wide range of cognitive domains, including executive functions, attention, working memory and speed of processing, in euthymic BD patients over a period of three months. At baseline, the authors found that BD patients showed significant deficits in speed of processing, attention and declarative memory compared to healthy individuals. In the

follow up, it was reported a significant interaction between change in neurpsychological performance and symptoms change scores in measures exploring the speed of processing as well as a trend towards significance in measures investigating executive functions and declarative memory. The authors suggested that increased in symptoms scores was associated with decreased task performance.

For manic BD patients, it was found that the manic state is dominated by poorer performances in verbal recognition memory (Martinez-Aran et al., 2004), episodic and working memory, spatial attention and problem solving (Sweeney at al., 2000) as well as sustained attention and verbal learning (Clark et al., 2001).

For depressed BD patients, it was suggested that the depressive state was characterized by poorer performances in visual immediate recall and phonemic fluency (Martine-Aran et al., 2004; Malhi et al., 2007), working memory (Sweeney et al., 2000), sustained attention and verbal learning (Malhi et al., 2007).

In summary, several strands of evidence from neuropsychological studies seem to support the presence of cognitive impairments in BD. The next paragraph provides an overview on the available evidence regarding the neural basis of these cognitive deficits in BD as examined using functional magnetic resonance imaging.

FMRI studies in BD

Working memory: The N-back

The N-back paradigm is one of the most extensively used paradigms for assessing working memory in fMRI studies investigating the neural activation elicited by increased memory load (1-,2-,3-back). In this task, the subject is presented with a sequence of stimuli, and the task consists of indicating when the current stimulus matches the one from *n* steps earlier in the sequence. The load factor *n* can be adjusted to make the task more or less difficult. This task engaged several brain regions including lateral and medial premotor cortex, dorsal cingulate, DLPFC, VLPFC, frontal poles, and posterior parietal cortex (Owen et al., 2005; Cremaschi et al., 2013).

Nine fMRI studies exploring the working memory circuit by using the N-back task in BD patients have been published so far, eight on euthymic BD patients (Adler et al., 2004; Drapier et al., 2008; Frangou et al., 2008; Hamilton et al., 2009; Jogia et al.,2012; Monks et al., 2004; Thermenos et al., 2010; Townsend et al., 2010) and one on manic BD patients (Pomarol-Clotet et al., 2013), all matched for age and sex with healthy individuals. The results of these fMRI studies showed a high degree of heterogeneity with regard to accuracy scores and neural response, especially for the 2-/3-back condition of the N-back task. For task performance, four studies showed significantly decreased accuracy scores in BD patients compared to healthy individuals in both 2-back (Pomarol-Clotet et al., 2013; Drapier et al., 2008; Thermenos et al., 2010) and 3-back (Drapier et al., 2008; Jogia et al., 2012) conditions.

With regard to neural activation, there was a general consistency indicating a dysfunctional activation in BD patients in the prefrontal cortex while performing the 2-back and 3-back condition. Specifically, the prefrontal areas that were found consistently altered in BD patients compared to healthy individuals included the DLPFC and the frontal pole (BA10). Four of the studies reviewed showed decreased activation of the DLPFC in BD patients suggesting the key role of the DLPFC in the pathophysiology of BD in respect of working memory processing (Hamilton et al., 2009; Monks et al., 2004; Pomarol-Clotet et al., 2013; Townsend et al., 2010). Although Hamilton et al. (2009) found no significant difference in the DLPFC in the direct comparison between BD patients and healthy individuals, the authors suggested that healthy individuals activated more the DLPFC compared to BD patients.

For the frontal pole, four studies have found the involvement of this area in BD patients. Depending on the N-back condition employed in these studies, BD patients showed increased (Adler et al., 2004; Drapier et al., 2008; Jogia et al., 2012) or decreased (Thermenos et al., 2010; Jogia et al., 2012) frontal pole activation compared to healthy individuals. Particularly, Drapier et al. (2008) was the only study that found the engagement of this area during processing of the 1-back condition, whereas all the others found it in either the 2-back (Adler et al., 2004; Jogia et al., 2012; Thermenos et al., 2010) or the 3-back (Jogia et al., 2012) conditions. Therefore, these differences may be due to the N-back condition employed in each single study and this mixed picture of hyper/hypo-activation

could be explained by the cortical inefficiency theory reported by Jogia et al. (2012).The authors showed that BD patients hyperactivated the frontal polar cortex during the 2-back condition without achieving optimal performance and hypoactivated it during the 3-back condition, suggesting the inability of patients to recruit this region beyond a certain cognitive load.

For the ACC, only one study by Monks et al. (2004) found decreased activation in BD patients compared to healthy individuals in the dorsal ACC (BA 24).

For parietal regions, four studies showed increased activation in BD patients compared to healthy individuals in parietal regions expanding from the inferior parietal lobule (BA 40) to the superior parietal lobule (BA 7) (Adler et al., 2004; Frangou et al., 2008; Drapier et al., 2008; Townsend et al., 2010). Only one fMRI study by Monks et al. (2004) reported decreased activation in the superior parietal lobule in BD patients compared to healthy individuals.

For temporal regions, three fMRI studies (Adler et al., 2004; Jogia et al., 2012; Monks et al., 2004) showed altered activation in BD patients compared to healthy individuals. Two of these studies reported increased activation in BD patients in the middle and superior temporal gyri (Adler et al., 2004; Jogia et al., 2012) whereas Monks et al. (2004) showed decreased activation in the middle temporal gyrus.

Finally, increased activation was also found in the anterior insula (Adler et al., 2004; Jogia et al., 2012; Thermenos et al., 2010), area associated with the limbic system and involved in emotional arousal (Critchley et al., 2000) and not normally activated during working memory tasks. However, after controlling for performance the hyperactivation of the insula in BD patients in the study by Adler et al. (2004) was not significant.

In summary, while performing this task BD patients failed to activate prefrontal regions which results in the engagement of several other brain regions, including areas within temporal and parietal cortex, in order to complete this cognitive demanding task.

Response Inhibition: the Stroop Task

The Stroop Test is a colour naming task used for assessing response inhibition. It is composed by three conditions: congruent, incongruent and neutral words. The neutral stimuli are those which are in the same text or colour as is displayed. In congruent stimuli colour and the colour name are the same. For instance, the word "RED" is written in "RED". The incongruent stimuli are those where the colour named by the word and the font colour do not match. For example, the word "RED" is written in "BLUE" font. In this task, the subjects were instructed to ignore the word referent and name the font colour (MacLeod et. al., 1991). It has been reported that the Stroop task engaged specific brain regions with the prefrontal and parietal cortex and ACC (Bush et al., 1998; Egner and Hirsch, 2005; Gruber et al., 2002).

Seven fMRI studies explored the neural activation of euthymic BD patients compared to healthy individuals by means of the Stroop task (Blumberg et al., 2003; Frangou et al., 2012; Gruber et al., 2004; Kronhaus et al., 2006; Pompei et al., 2011; Roth et al., 2006; Strakowski et al., 2005). Additionally, Blumberg et al. (2003) is the only study that included results related to BD patients also in acute manic and depressed state.

Two versions of the Stroop task have been used in these studies: the counting Stroop and the colour-word naming Stroop tasks. With regard to task performance, all fMRI studies except for Strakowski et al. (2005) showed no difference in accuracy scores of BD patients compared to healthy individuals. For reaction time, two studies showed that BD patient were significantly slower in completing the interference condition compared to healthy individuals (Gruber et al., 2004; Kronhaus et al., 2006).

On the neuronal level, the majority of these studies found evidence of hypoactivation in prefrontal regions in BD patients compared to healthy individuals, including the VLPFC, DLPFC, and the ACC.

The decreased activation of the VLPFC in euthymic BD patients is the most consistent finding across all of the reviewed studies (Blumberg et al., 2003; Kronhaus et al., 2006; Pompei et al., 2011; Roth et al., 2006). The study by Blumberg et al. (2003) also showed that depressed BD patients had increased activation in the left VLPFC and manic patients showed blunted

response in the right VLPFC.Similarly, decreased activation was also found in BD patients compared to healthy individuals in subdivision of the cingulate cortex, including the posterior cingulate cortex (Roth et al., 2006) and the perigenual ACC (Gruber et al., 2004) during processing of the counting Stroop and the colour-word naming Stroop tasks.

For DLPFC, two studies (Gruber et al., 2004; Kronhaus et al., 2006) showed opposite results with increased (Gruber et al., 2004) and decreased (Kronhaus et al., 2006) activation of the DLPFC.

In summary, overall these studies suggest that BD patients showed decreased prefrontal and cingulate cortex activation compared to healthy individuals. Moreover, the only study that explored the neural activation during the Stroop task in BD patients in different affective states suggested that the decreased activation of the VLPFC is a common feature in euthymic and depressed but not in manic BD patients. However, more studies are needed for further replicate these results.

Executive functions: Verbal Fluency task

Three fMRI studies were carried out in euthymic BD patients during the performance of the phonological letter verbal fluency task which consists in generating a word beginning with a cue letter (Allin et al., 2010, Costafreda et al., 2011, Curtis et al., 2001). This task engages several brain regions, including the ACC, left middle and inferior frontal gyri, parietal cortex, and right cerebellum (Fu et al., 2002). Allin et al. (2010) employed two easy and hard sets of letter and showed that in both conditions BD patients had increased activation of the posterior cingulate cortex (PCC) compared to healthy individuals. Moreover, for the easy conditions they also reported decreased activation in the frontal cortex in BD patients. The authors found a significant difference in performance, where BD patients made more errors in the easy but not in the hard condition. Curtis et al. (2011) found increased activation in the superior medial parietal cortex, medial frontal cortex, cerebellum, lingual gyrus and inferior frontal gyrus in BD patients compared to healthy individuals. Finally, Costafreda et al. (2001) showed increased activation in the ACC, DLPFC and putamen as well as decreased activation in the precuneus and PCC in BD patients compared to healthy individuals. No significant difference in performance has been found in both studies.

Sustained attention: the Continuous Performance Task (CPT)

The CPT involves continual monitoring of the information through visual attention. The task may use number, symbols or sounds and it engages different brain regions, including frontal and temporoparietal cortex, ACC and insula (Sepede et al., 2014).

Three fMRI studies investigating the sustained attention in BD patients while performing a continuous performance test (CPT) in either manic (Fleck et al., 2012) or euthymic (Sepede et al., 2012; Strakowski et al., 2004) have been published so far. These studies have consistently reported altered activation in BD patients in the inferior frontal gyrus/insula with increased activation found by Strakowski et al. (2004) and reduced activation reported by Fleck et al. (2012). Sepede et al. (2012) reported both decreased and increased activation in this area during processing of correct or wrong target responses respectively. Two studies showed increased activation of the amygdala in both euthymic (Strakowski et al., 2004) and manic (Fleck et al., 2012) BD patients.

2.3 Reliability of the N-back and the Facial Affect Labelling tasks

Although the N-back and the facial affect labelling tasks have become a standard for investigating working memory and emotional processing respectively, it is vital to explore their construct validity and test-retest reliability. For the N-back, the behavioural study by Hockey et al. (2004) reported test-retest reliability measures of a visuospatial version of the N-back task at four levels of load (0-3-back) and yielded reliability measures between r= 0.49 (1-back) and r= 0.73 (3-back) for accuracy, and even better values for reaction times (r= 0.69 (2-back) and r= 0.86 (0-back). For the facial affect labelling task, behavioural studies reported that the test-retest reliability of facial emotional stimuli depends on the clarity of emotion expression (Rojhan et al., 2000; Limbrecht et al., 2012). These studies have shown that happy and sad items were easier to agree upon than neutral ones and that happy items had the highest validity, highest test–retest reliability, and highest item-total correlations.
Moreover, the study by Plichta et al. (2012) investigated both within-subject and group-level reliability of brain regions associated with the N-back and facial emotion recognition task in a group of healthy individuals that have been scanned twice on an MRI scanner. The overall results showed a robust activation of the two tasks in their respective target regions (emotional task=amygdala, working memory task=DLPFC and parietal cortex). Moreover,

the reliability of group level activation was excellent for the two tasks, with intraclass correlation coefficient of 0.89–0.98 at the whole brain level and 0.66–0.97 within target ROIs.

2.4 Conclusion

In conclusion, anatomical studies have consistently shown a ventricular enlargement in BD patients compared to healthy individuals but contrasting and limited findings in regards to GM volumes changes in cortical and subcortical regions have been reported.

However, evidence from fMRI and neurpsychological studies in affective and non-affective cognition suggest that BD patients show extensive neurocognitive deficits as well as dysfunctions in several cortical and subcortical brain regions. For the affective cognition, the majority of fMRI studies in euthymic BD patients report an overall impairment in frontolimbic regions across all the emotional tasks described above; with hypoactivation of the dorsal system including the VLPFC and DLPFC and hyperactivation of the ventral system including the amygdala. On the other hand, for manic and depressed BD patients, most of the evidence originates from studies that employed facial emotion recognition tasks. These studies suggest increased activation of the amygdala in both manic and depressed BD patients as well as reduced prefrontal activation, including VLPFC and DLPFC, in depressed BD patients compared to healthy individuals in response to affective faces. For the non-affective cognition, the majority of the fMRI studies use euthymic BD patients as a sample. Overall, these studies show dysfunctional activation in several cortical components including the DLPFC, VLPFC and ACC; with decreased activation in these areas in euthymic BD patients across the majority of cognitive tasks described. Interestingly, evidence from the few fMRI studies investigating manic BD patients suggest the same general reduction in prefrontal regions and specifically within the VLPFC and DLPFC.

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3. Neural correlates of emotional processing in Bipolar Disorder: a voxel-based meta-analysis of functional magnetic resonance imaging studies

This chapter aims to synthesize the evidence from two published meta-analyses investigating facial affect processing network in Bipolar Disorder (BD)(Delvecchio et al., 2012; Delvecchio et al., 2013, published copies available in the Appendix) and discusses the common and distinct pattern of neural activation between BD, Major Depressive Disorder (MDD) and Schizophrenia (SZ).

3.1 Introduction

Our understanding of the neural circuitry involved in emotional processing in healthy individuals is mostly based on studies using facial affect as a probe (Phan et al., 2002; Murphy et al., 2003; Fusar-Poli et al., 2009; Vytal and Hamann, 2010). Facial affect processing engages numerous neural systems with key nodes spatially distributed within the primary and extrastriate visual cortices (particularly within the fusiform gyrus) in the occipital and ventral temporal lobes, the superior temporal sulcus, the medial and ventrolateral prefrontal cortex (PFC), limbic (amygdala and insula, among others) and thalamic areas (Adolphs et al., 1996; Haxby et al., 2002; Phan et al., 2002; Murphy et al., 2003; Fairhall & Ishai, 2007; Fusar-Poli et al., 2009; Vytal & Hammann, 2010; Dima et al., 2011). We used Activation Likelihood Estimation (ALE) (Turkeltaub et al., 2002; Laird et al., 2005; Eickhoff et al., 2009), a quantitative meta-analytic approach which allows integration of neuroimaging results across studies, to investigate the neural correlates of facial affect processing in BD and compared them to those of MDD and SZ.

Recurrent depressive episodes and similar subsyndromal affective symptoms are shared features of MDD and BD (Judd et al., 2002, 2003; Angst et al., 2010). Evidence from genetic studies suggests both distinct and common contributions to their aetiology (McGuffin et al., 2003). Current neurobiological models propose that mood disorders arise from disruption in prefrontal, limbic and subcortical regions (particularly the amygdala/hippocampus, and striatum) that support the adaptive regulation of affect (Savitz and Drevets, 2009). Within this general framework, much research effort in neuroimaging is directed towards identifying overlapping and diagnosis-specific brain abnormalities for BD and MDD. Several

reviews and meta-analyses have attempted to summarise and synthesise the available evidence. For example, the most recent quantitative meta-analysis of structural magnetic resonance studies (Kempton et al., 2011) showed that volume reductions in the basal ganglia and hippocampus appear specific to MDD patients and differentiated MDD from BD.

BD and SZ also show significant overlap in terms of clinical symptoms (Fischer & Carpenter, 2009), genetic risk factors (International Schizophrenia Consortium, 2009; Lichtenstein et al., 2009) and brain morphological changes (Ellison-Wright & Bullmore, 2010; Yu et al., 2010). Specifically, the reason whywe focused specifically on facial affect processing and we examined whether diagnosis-related differences in the engagement of the corresponding neural network support a distinction between SZ and BD are threefold.

First, differences in emotional processing have been highlighted as the most distinctive features differentiating SZ from BD. Both early descriptions and later investigations of the two syndromes (Bleuler, 1950; Kraepelin, 1971; Carpenter et al., 1973) emphasize avolition and restricted affect in SZ and excess emotional reactivity in BD.

Second, most of the regions involved facial affect processing are also implicated in current models of BD and SZ that respectively highlight abnormal interaction within prefrontal–subcortical–limbic (Strakowski et al., 2005) and prefrontal–temporal–subcortical–limbic networks (Gur et al., 2007a).

Third, available findings in BD and SZ are suggestive of diagnosis-specific differences, particularly within limbic and posterior parieto-occipital regions. For example, viewing emotional faces compared to neutral faces is associated with increased engagement of the parahippocampus/amygdala in BD (Altshuler et al., 2005; Strakowski et al., 2005; Chen et al., 2006; Foland et al., 2008; Delvecchio et al., 2012) but not in SZ (Aleman & Kahn, 2005; Holt et al., 2006; Surguladze et al., 2006; Hall et al., 2008; Seiferth et al., 2009; Li et al., 2010; Anticevic et al., 2012). Additionally, recruitment in medial occipital and parietal regions involved in visual processing has been reported to be enhanced in SZ (Farkas et al., 1984; Taylor et al., 2012) and reduced in BD (Pavuluri & Passarotti, 2008).

However, inferring potential differences or similarities between the BD, MDD and SZ from existing studies is difficult because direct comparisons between the diagnostic groups are

limited. Therefore, meta-analytic techniques are currently the best option to investigate brain regions differentially engaged in BD, MDD and SZ, and thus address questions of diagnostic specificity.

The main goals are threefold. First, to consolidate neuroimaging findings associated with emotional processing in patients with BD and to examine whether meta-analytic synthesis of this empirical evidence aligns with current theoretical models of BD (Cerullo et al., 2009; Savitz and Drevets, 2009). Second, to determine whether stimulus valence modulates disease-related activity within the face processing network in BD patients. Third, to describe the distinct and common patterns of activation between BD, MDD and SZ.

3.2 Methods

3.2.1 Data sources and inclusion criteria contrasting BD to MDD and SZ

Studies investigating facial affect processing in BD patients, MDD and SZ were identified through a comprehensive MEDLINE, EMBASE and PsycINFO search of the English-language literature covering publications between January 2000 and April 2012. The search keywords were “mania ”, “depression ”, “bipolar disorder”, " schizophrenia", “emotional processing”, "facial affect labelling", "facial affect matching", “fMRI” and their combinations as well as terms specifying individual facial affect (fear, happiness, sadness, anger and disgust). Additional articles were identified through the reference lists of these papers.

Studies were included if they (a) reported comparisons between patients with BD, MDD or SZ with healthy individuals (b) employed functional magnetic resonance imaging (fMRI) (c) assessed brain activation by using human facial identities (d) used image subtraction methodology to identify foci of task-related neural changes contrasting an active (emotional faces) and control (neutral faces or shapes) condition, and (e) reported their results in standard stereotactic coordinates (either Talairach or Montreal Neurological Institute [MNI] space).

We excluded studies that (a) used facial affect stimuli to investigate processes not directly involved in emotional processing (e.g. memory, attention), (b) involved non-facial identities such as emotional pictures, (c) grouped together stimuli displaying positive and negative facial affect, and (d) used the same patient sample e) focused on pediatric or geriatric

patients. The threshold of statistical inference varied but we accepted the results reported as significant based on the criteria of the primary studies.

3.2.2 Quantitative meta-analytical voxel-based procedure

Facial affect processing was examined based on the contrast between facial affect and control conditions using Activation Likelihood Estimation (ALE) implemented in GingerALE 2.0.4 (http://brainmap.org/Ale). The ALE method is a meta-analytical approach to neuroimaging that attempts to assess above chance clustering of activation probabilities across studies. In other words, the aim of ALE is to determine where overlap across the fMRI studies is significantly higher than expected when results are independently distributed (Eickhoff et al., 2009). In the ALE, the data imputed represent significant BOLD activation signals. The key advantage of ALE is that it has a neurobiologically and mathematically stringent concept based on probabilistic inference about activation coordinates. Instead of trying to model the activation map or even effect sizes, ALE coherently only deals with activation coordinates, which are represented in a probabilistic fashion to account for spatial uncertainty. This ALE version uses a random effect model and weighting for sample size of the original studies (Eickhoff et al., 2009). The statistical approach used in these meta-analyses involved several steps. Firstly, coordinates of the foci of activation reported in the primary literature were transformed into Talairach space using the Lancaster transform (icbm2tal tool) in GingerALE. Secondly, smoothing took place, in which ALE generated a Gaussian probability distribution around the cluster coordinates reported in fMRI studies (Turkeltaub et al., 2002). Thirdly, whole brain modelled activation (MA) maps were generated in order to provide an estimate of the likelihood that any given voxel was involved in the disorder under investigation. ALE scores from the convergent MA maps were then calculated on a voxel-by-voxel basis to test for convergent (random-effects) rather than study specific foci (fixed-effects). Following, False Discovery Rate (FDR) technique was applied to define a threshold of significance (Laird et al., 2005). Neighbouring voxels that were marked as significant were grouped together as resultant clusters and the smaller clusters were filtered out to avoid noises in the result. Importantly, the foci that served as the input for the analyses were weighted by the number of participants in each study to assign more weight to the studies that carry more information. A similar approach for weighting the studies consists in using the inverse of the variance rather than the sample

size. However, this approach is roughly proportional to sample size, but is a more nuanced measure, and serves to minimize the variance of the combined effect. Finally, an important drawback of the ALE method is that there is no weighting of foci by significance, design or demographic and clinical variables. However, the ALE algorithm identifies common activations, and thereby factors out effects not related to the process of interest, such as different methodologies that are used by different research groups. Moreover, in my two meta-analyses most of the fMRI studies included were age and gender matched thus minimising the likelihood of a systematic sex- and age-related bias confounding the effect of diagnosis. With regard to clinical and behavioural variables, the ALE algorithm was not able to adequately address the impact of these variables on the results. Such questions would be ideally investigated in large neuroimaging data sets where the quantitative impact of these variables could be directly assessed (e.g. by including only BD or MDD patients in remission or in acute phase of the illness). All ALE data processing was performed using the BrainMap Search and View software (http://brainmap.org).

First, three separate global meta-analyses were performed (a) all studies comparing BD patients to healthy individuals, (b) all studies comparing MDD patients to healthy individuals, and (c) all studies comparing BD to SZ patients. Differences between diagnostic groups were tested by computing the voxel-wise difference between the ensuing ALE maps. Statistical inference was based on a threshold of $p< 0.05$ with False Discovery Rate (FDR) correction and a minimum cluster size of 200 mm^3.

At a second stage, a series of subsidiary meta-analyses were conducted, based on data availability only for patients with mood disorders, focusing on fearful and happy facial expressions as exemplars of negative and positive valence. For all analyses, all ALE maps were imported into Mango and overlaid on an anatomical template (http://ric.uthscsa.edu/mango/) for representation purposes. Coordinates of the maximum ALE and corresponding Brodmann areas are reported.

Moderator variables for the SZ and BD group comparison

Demographic information about age and sex and clinical information about level of psychopathology and medication type and dosage were extracted from each primary study.

Mean antipsychotic dose was converted to chlorpromazine equivalents (Bezchlibnyk-Butler & Jeffries, 2010). The effect of other medications (e.g. lithium) was not examined because dosage was not mentioned in a sufficient number of studies. We extracted information about participants' scores on the Hamilton Depression Rating Scale (HAMD; Hamilton, 1960), the Young Mania Rating Scale (YMRS; Young et al., 1978) and the positive and negative subscales of the Positive and Negative Syndrome Scale (PANSS; Kay et al., 1987). We tested for potential group differences in these moderator variables and examined their influence on the ALE results using meta-regression analyses. The impact of symptom severity was examined in each diagnostic group separately. Based on procedures implemented in previous studies (Van Snellenberg et al., 2006; Anticevic et al., 2012), we rescaled each psychopathology scale from 0 to 1, where 0 and 1 indicated respectively the minimum and maximum possible scores on each scale.

3.3 Results contrasting BD to MDD

We identified 37 studies that used facial affect paradigms in patients with BD or MDD of which twenty fulfilled all inclusion criteria, giving a total sample of 168 BD and 189 MDD patients and 344 healthy individuals (Table 3-1 and Table 3-3). Excluded studies (a) grouped mood disorder patients together with other diagnostic groups (Lau et al., 2009), (b) grouped positive and negative facial stimuli together (Matthews et al., 2008; Yang et al., 2010; Anderson et al., 2011), (c) did not provide coordinates of the case – control comparison (Yurgelun-Todd et al., 2000; Gaffrey et al., 2010; Liu et al., 2010; Versace et al., 2010) or of the emotional versus neutral facial expressions contrast (Victor et al., 2010; Frodl et al., 2010), (d) did not include a control group (Keedwell et al., 2009), (e) implemented functional connectivity (Almeida et al., 2009) or pattern classification analyses (Fu et al., 2008), (f) used facial affect stimuli to examine other processes (e.g. interference) (Keedwell et al., 2005; Fales et al., 2008), and (g) examined the same patient group as other included studies (Haldane et al., 2008; Fu et al., 2004).

Demographic details for all participants and clinical information about BD and MDD patients in the studies included are shown in Table 3-1, Table 3-3, Table 3-4 and Table 3-6. As the definitions of participants' mental state differed in the primary studies we provided the mean psychopathology rating scales' scores (Table 3-4 and 3-6). With the exception of 5

studies which included medication free patients, patients received combinations of different psychotropics.

3.3.1 Facial Affect processing abnormalities in BD compared to healthy individuals

BD patients compared to healthy individuals showed (a) increased activation in the parahippocampal gyrus (extending to the amygdala) bilaterally, in the left putamen and left pulvinar (14 studies; 50 foci; 379 subjects) (Table 3-7), and (b) decreased activation bilaterally in the ventrolateral prefrontal cortex, within the inferior frontal gyrus (BA47) (Table 3-7) (Fig. 3-1).

There was evidence of valence specific differentiation in the facial affect network engagement in BD patients compared to healthy individuals with respect to fearful and happy facial stimuli.

In the fearful > non emotional stimuli contrast BD patients, compared to healthy individuals, showed (a) increased activation in the left parahippocampal gyrus (BA 28/35), left putamen and left pulvinar thalamus (7 studies; 30 foci; 166 subjects), and (b) decreased activation in the inferior frontal gyrus (BA 47/45) bilaterally and in the left anterior cingulate gyrus (BA32) (7 studies; 23 foci; 170 subjects) (Table 3-8) (Fig. 3-1).

With respect to the happy > non emotional stimuli contrast BD patients compared to healthy individuals (a) increased activation in the caudate bilaterally and left parahippocampal gyrus (BA 34) (4 studies; 16 foci; 132 subjects) and (b) decreased activation in the right anterior cingulate gyrus (BA 32) (3 studies; 23 foci; 95 subjects) (Table 3-8) (Fig. 3-1).

3.3.2 Facial Affect processing abnormalities in MDD compared to healthy individuals

MDD patients compared to healthy individuals showed (a) increased activation in the right parahippocampal gyrus (extending to the amygdala) (7 studies; 24 foci; 280 subjects) (Table 3-9), and (b) decreased activation in the right putamen, and left caudate (10 studies; 44 foci; 423 subjects) (Table 3-9) (Fig. 3-2).

There was evidence of valence specific differentiation in the facial affect network engagement in MDD compared to healthy individuals with respect to fearful and happy facial stimuli.

In the fearful > non emotional stimuli contrast MDD patients showed decreased activation in sensorimotor cortices within the left precentral gyrus (BA 6) (4 studies; 14 foci; 97 subjects) (Table 3-9)(Fig. 3-2).

With respect to the happy > non emotional stimuli contrast, activation was decreased in the right pulvinar thalamus in MDD patients compared to healthy individuals (Table 3-9)(Fig. 3-2).

3.3.3 Facial Affect processing abnormalities in BD compared to MDD

BD patients showed greater likelihood of activation than MDD patients in the parahippocampal gyrus (cluster included the amygdala), in the ventral anterior cingulate gyrus bilaterally and in the left pulvinar. Conversely, MDD patients had increased likelihood of activation than BD patients in dorsal anterior cingulate gyrus (Table 3-9) (Fig. 3-2).

3.4 Results contrasting BD to SZ

The study selection flowchart is shown in the PRISMA diagram provided in Fig. 3-S1. We included 29 studies that fulfilled all inclusion criteria, giving a total sample of 268 patients with SZ, 267 BD patients and 483 healthy individuals. Demographic details for all participants and clinical information of SZ and BD patients are shown in Table 3-1, Table 3-2, Table 3-4 and Table 3-5. There was a significant difference between the two patient groups in age (SZ: mean=31.69 years, Standard Deviation (SD)= 6.1 years; BD: mean= 35.9 years, SD= 4.1; t=-2.33 p= 0.026) but not sex (SZ: 64.3% men, 35.7% women, BD: 56.2% men, 43.8% women, χ^2= 3.55, p= 0.06).

3.4.1 Facial Affect processing abnormalities in BD compared to healthy individuals

For this analysis we included two additional studies that have not been considered in the analyses contrasting BD to MDD. The results of this analysis are the same as described in the paragraph 3.3.1 except for the left anterior cingulate cortex (BA 32, x=-10, y=30, z=18, cluster size= 200, ALE-value= 0.01) and the right caudate (x=28, y=26, z=8, cluster size=

2368, ALE-value= 0.01) where BD patients showed decreased activation compared to healthy individuals.

3.4.2 Facial Affect processing abnormalities in SZ compared to healthy individuals

Compared to healthy individuals, patients with SZ showed an increased likelihood of activation in the right cuneus and a decreased likelihood of activation in frontal regions [left precentral gyrus (BA 6) and left medial frontal gyrus (BA 9)], in limbic and paralimbic regions [right amygdala, right insula and left parahippocampal gyrus (BA 28) and anterior cingulate cortex (ACC; BA 32)], in occipital and occipito-temporal regions [right inferior occipital gyrus (BA 17) and right fusiform gyrus (BA 37)], in the basal ganglia (right caudate nucleus) and in the right medial dorsal thalamus (19 studies; 103 foci and 645 subjects).Details are shown in Table 3-10 and Fig. 3-3. No effect of gender was observed but age was positively correlated with the likelihood of activation in the left parahippocampal gyrus (BA 28) (x=20, y=26, z=8; r^2= 0.66, p= 0.001, voxels= 227). A higher PANSS positive symptoms subscale score correlated with a reduced likelihood of activation in the hippocampus/parahippocampal gyrus (x=38, y=18, z=14; r^2= 0.56; p= 0.001, voxels= 619). A higher PANSS negative symptoms subscale score correlated with a reduced likelihood of activation in the precentral gyrus (BA 6) (x=38, y=2, z=40; r^2= 0.56, p= 0.001, voxels= 33).

3.4.3 Facial Affect processing abnormalities in SZ compared to BD

Compared to SZ patients, patients with BD were more likely to activate the left pulvinar thalamus (Table 3-10, Fig. 3-3). Conversely, patients with SZ were more likely to activate the cuneus bilaterally (BA 18) (Table 3-10, Fig. 3-3). Age and sex did not contribute to differences between diagnostic groups. Differences between the two disorders in amygdala activation were negatively correlated with antipsychotic dose (x=22, y=6, z=12; r^2= 0.98, p= 0.001, voxels= 832).

3.5 Discussion

These results provide evidence of the common and distinct patterns of activation between BD, MDD and SZ that can be summarized in seven key findings. First, both BD and MDD patients showed increased activation in limbic regions irrespective of stimulus valence while SZ patients showed an opposite pattern of activation, with decreased limbic activation

compared to healthy individuals. Second, BD and SZ was associated with reduced prefrontal cortical activation. Third, MDD showed decreased engagement of somatosensory cortices compared to healthy individuals. Fourth, activation in the pulvinar thalamus and basal ganglia was increased in BD compared to healthy individuals and MDD patients. Fifth, these findings showed evidence of modulation by stimulus valence. Sixth, SZ patients showed increased likelihood of activation in the cuneus (and adjacent posterior cortical regions) compared to healthy individuals. Seventh, additional differences were found when BD and SZ patients were compared to each other. BD patients showed greater likelihood of activation in thalamic regions compared to SZ patients; in the reverse comparison, SZ patients showed increased likelihood of activation in the posterior visual association cortices.

3.5.1 Increased limbic engagement: a common feature of BD and MDD but not SZ

Evidence in BD and MDD

These findings broadly confirm the prevailing view that mood disorders are associated with increased limbic activation during emotional processing (Savitz and Drevets, 2009). These data question however the current “amygdalocentric ” models for mood disorders. Clusters of abnormal medial temporal activation in both BD and MDD centred on the parahippocampal gyrus although they extended to include the amygdala. Chen et al. (2011) reported a similar pattern in a previous meta-analysis of fMRI studies in BD (Chen et al., 2011) and we now extend these observations to MDD. The parahippocampal gyrus and amygdala lie very close to each other and frequently co-activate during emotional processing (Fusar-Poli et al., 2009; Vytal and Hamann, 2010). Therefore the accuracy in locating the peak of activation within medial lobe structures could be influenced by smoothing and transforming data from individual subjects into a common stereotactic space. Other methodological considerations may relate to the type of paradigm or mood state. For example the amygdala become more engaged when facial expressions are processed outside the focus of attention (Hariri et al., 2003) and their activation may “normalise” with symptomatic remission, especially in MDD (Delaveau et al., 2011). Alternatively, it is possible that these results genuinely reflect greater parahippocampal involvement, relative to the amygdala, in mood disorders. The parahippocampal gyrus and

the amygdala are thought to subserve partially segregated dimensions of emotional processing; amygdala engagement may signal salience or ambiguity (Gerber et al., 2008; Santos et al., 2010) while parahippocampal activation may reflect context appraisal (Gerdes et al., 2010). Whether these processes are differentially affected in mood disorders requires further investigation. In any case these results add to the emerging consensus that a more detailed evaluation of the role of limbic structures in mood disorders is warranted and this should crucially involve a revaluation of the central role currently ascribed to the amygdala. This is timely as within the field of affective neuroscience the role of the amygdala is undergoing major reappraisal with greater emphasis being placed on the contribution of other cortical and subcortical structures (Pessoa and Adolphs, 2010).

Evidence in SZ

On the other hand, in the contrast between emotional and neutral facial expressions, patients with SZ show the opposite pattern of activation observed in mood disorders, with reduced activation within the amygdala/parahippocampus compared to both BD patients and healthy individuals. This has been attributed to abnormally elevated responses to neutral facial stimuli (Aleman & Kahn, 2005; Holt et al., 2006; Surguladze et al., 2006; Hall et al., 2008; Anticevic et al., 2012) and is thought to reflect abnormal salience attribution (Kapur, 2003). However, Gur et al. (2007b) reported that limbic activation was abnormally elevated in patients with SZ when they failed to identify facial expressions. This information, combined with behavioural evidence that SZ is associated with deficits in correct facial affect identification, suggests that patients find these stimuli ambiguous regardless of valence (Pinkham et al., 2007). It could therefore be argued that it is ambiguity (Santos et al., 2010), rather than inappropriate attribution, that drives limbic recruitment in SZ.

3.5.2 Prefrontal cortex involvement: a common feature in BD and SZ but not MDD

BD, but not MDD, was associated with reduced engagement in ventrolateral prefrontal regions within the inferior frontal gyrus. The ventrolateral prefrontal cortex is involved in inhibitory control across a number of paradigms including emotional processing (Quirk and Beer, 2006). Dysfunction within this region is therefore thought to reflect reduced inhibitory capacity in BD (Cerullo et al., 2009; Chen et al., 2011). The degree of dysfunction in this region may be modulated by valence as it was most consistently observed when BD patients

processed negative facial expressions. Ventrolateral PFC engagement regulates stimulus-driven action by modulating the influence of emotional stimuli on cognition with respect to contextually (or socially) appropriate behaviour (Quirk and Beer, 2006). In this respect ventrolateral PFC dysfunction may be relevant to stimulus-driven, socially inappropriate behaviour observed during mania. Similarly, SZ patients showed reduced activation in the dorsolateral prefrontal regions within the medial PFC compared to healthy individuals. The dorsolateral PFC modulates the generation, interpretation and regulation or identification of emotions and it is involved in cognitive control processes (Ochsner et al., 2005; Lesh et al., 2011). This finding is in line with previous fMRI evidence showing a generalized hypofrontality in SZ patients in this region while viewing negative emotional stimuli (Williams et al., 2007; Takahashi et al., 2004). Given the role of the dorsal PFC in the integration of cognitive mechanisms with brain regions associated with emotion (Lesh et al., 2011), a dysfunction within this region may be therefore related to dysfunctional top-down support from cognitive control processes.

3.5.3 Increased thalamic engagement in BD compared to MDD and SZ

Increased thalamic engagement was uniquely associated with BD, which indicates enhanced attention towards emotionally salient stimuli from the early stages of visual processing (Pessoa & Adolphs, 2010). However, the results in BD and MDD suggest that thalamic involvement in mood disorders is complex. Thalamic activation may be influenced by stimulus valence. Increased pulvinar activation was observed in BD during the processing of negative facial expressions while decreased activation in the same nucleus was noted in MDD during the processing of happy faces. The pulvinar is considered a “higher order” nucleus because of its widespread bidirectional cortical connections (Pessoa and Adolphs, 2010). As mentioned above, the pulvinar is directly involved in visual perception (Pessoa and Adolphs, 2010), particularly in directing and maintaining attention towards salient stimuli (Desimone et al., 1990). Therefore, these results suggest that in BD the pulvinar overactivation may act to amplify neural engagement for emotionally salient, particularly negative stimuli. The reverse appears to be the case in MDD when processing happy faces; this is in line with current views that MDD may be characterised by reduced reactivity to positive stimuli (Rottenberg et al., 2005).

3.5.4 Distinct basal ganglia involvement in BD and MDD

Diagnosis related changes were also noted in the basal ganglia where increased engagement was observed in BD compared to healthy individuals while the reverse was the case for MDD. Specifically, BD patients expressed increased activation in the putamen and in the caudate in response to negative and positive facial expressions respectively. The putamen is mainly involved in sensorimotor processing and is thought to contribute to the motor production of facial expressions during negative affect recognition (Adolphs et al., 2002). Increased putamen activation in BD patients may reflect either greater facial mimicry or greater amplification of sensorimotor processing of negative facial affect. The latter interpretation is supported by the increased engagement of the pulvinar. During happy facial affect processing BD patients, compared to healthy individuals, expressed increased activation in the caudate nucleus. This is in line with findings implicating the caudate in processing rewarding stimuli (Schultz et al., 1997; O'Doherty et al., 2003) including happy facial expressions (Phan et al., 2002). As activation in reward-circuitry structures correlates positively with valence (Gerdes et al., 2010) these results indicate that happy facial stimuli may have greater reward value for BD patients. This observation may relate to the inappropriate and generalised activation of reward-related structures in mania (Abler et al., 2008).

3.5.5 Somatosensory corteces involvement: a unique feature of MDD

MDD patients showed decreased responsiveness in the somatosensoty corteces compared to healthy individuals, particularly when viewing negative facial expressions. Somatosensory cortices contribute to the recognition of facial emotions (Adolphs et al., 2002) possibly through a process of invoking or “ mirroring ” internal representations of the pertinent emotional experience (Adolphs et al., 2000). Since MDD patients experience negative emotions frequently as part of the clinical syndrome of depression this finding could be suggestive of adaptive down-regulation of processing of negative stimuli. Similar observations of reduced emotional reactivity in MDD have been made previously in a variety of experimental settings and are thought to reflect emotion-context insensitivity (Rottenberg et al., 2005).

3.5.6 Visual cortex involvement may typify SZ but not BD and MDD

SZ was associated with increased likelihood of engagement within the cuneus compared to healthy individuals and patients with BD (Table 3-10). This was coupled to reduced engagement within the occipitotemporal cortices, including the inferior occipital and fusiform gyrus, compared to healthy individuals. The occipito-temporal cortices are involved in early stimulus categorization (Pizzagalli et al., 2002; Lachaux et al., 2005; Tsuchiya et al., 2008) and therefore this cluster of hypo-activation in these areas in SZ may suggest an impairment in visual processing of facial stimuli (regardless of emotional expression) from the early stages of sensory perception (Chen et al., 2009). Difficulties in stimulus categorization are also likely to increase stimulus ambiguity. It is therefore possible that a more general dysfunction in visual perception contributes to abnormal facial affect processing in SZ (Kumar et al., 2010; Green et al., 2011).

The cluster of overactivation identified corresponds to the medial cuneus, which is part of the extrastriate visual cortex (Iaria et al., 2008) and thus involved in higher-order processing of visual information. The function most consistently ascribed to the cuneus relates to early stimulus categorization. In this respect the cuneus may enlist mnemonic and/ or attentional mechanisms towards features that distinguish categories and thus modulate the quality or quantity of visual information reaching later processing stages (Sergent et al., 1992; Vanni et al., 2001). This pattern of overactivation within higher-order visual cortices in SZ was also observed by Taylor et al. (2012) in a meta-analysis of fMRI studies of emotional perception and by Seiferth et al. (2009) in a study of facial affect processing in adolescents with SZ.

Taken together, these findings suggest a significant role for the visual cortices in SZ that is probably already present at the earliest stages of the illness. The nature of this overactivation within higher-order visual regions in SZ is unclear but the prevailing view is that it represents a 'compensatory' response to deficits in integrating visual information (Seiferth et al., 2009; Taylor et al., 2012). Visual abnormalities in SZ are not limited to the perception of facial expressions but have been reported in multiple paradigms including motion and form perception, spatial frequency and location discrimination, perceptual organization and backward masking (Butler et al., 2008; Kumar et al., 2010; Green et al., 2011).

3.6 Methodological considerations

Activation Likelihood Estimation represents a powerful approach for the meta-analytic treatment of neuroimaging data. Still, a number of factors should be considered in the interpretation of the current set of findings.

First, this initial review revealed great variability in the emotional processing paradigms used which coupled with small sample sizes impacts on the ability to draw statistically robust conclusions from this literature. To minimise variability due to study design we focused exclusively on studies using comparable versions of facial affect processing tasks.

Second, we included results reported as significant in the original studies since ALE analyses do not allow weighting based on the threshold of significance employed in each individual study.

Third, there was significant variability in the level of patients' symptomatology at the time of testing and in the definition of “remitted” or “euthymic” states (Table 3-4, Table 3-5 and Table 3-6). Separate analyses of the available studies according to mood states would not have been statistically feasible. Therefore, the changes in regional brain activity identified here cannot be clearly categorised as trait or state.

Fourth, as shown in Table 3-4, Table 3-5 and Table 3-6, with the exception of five studies, patients were medicated and were prescribed combinations of psychotropics. Given the inter-study variability in medication regimes a systematic bias influencing these results is improbable. Additionally psychotropic medication predominantly acts to reduce case–control differences in neural activity in mood disorders (Phillips et al., 2008; Delaveau et al., 2011) and schizophrenia (Cabral-Calderin et al., 2010). In the study by Cabral-Calderin et al (2010) antipsychotic dose seemed to reduce group differences in amygdala/ parahippocampal engagement. Antipsychotic medication is known to influence the function of the amygdala (Aleman & Kahn, 2005) but the effect observed here suggests that it minimizes diagnostic differences.

Fifth, sex differences have been found during facial affect processing (Fusar-Poli et al., 2009). Although it was not possible to examine this directly, the original studies included samples that were generally balanced and matched for sex (Table 3-1, Table 3-2,Table 3-3),

thus minimising the likelihood of a systematic sex-related bias confounding the effect of diagnosis.

Sixth, since most original studies examined multi-episode BD patients (Table 3-4), further investigation is required to clarify the relevance of these results to the initial stages of mood disorders.

Seven, current meta-analytic algorithms cannot adequately address the effect of demographic, behavioural or clinical variables on the distribution of the reported brain activation patterns. Such questions would be ideally investigated in large neuroimaging data sets where the quantitative impact of these variables could be directly assessed.

Eight, a further limitation is the variability in the types of tasks used in individual source studies, which precludes separate analyses per task type because of small sets sizes. As the primary literature expands, more task-specific meta-analyses should be possible in the future. However, these results achieved a degree of robustness that suggests that we captured the most replicable differences between patients with BD, MDD or SZ.

Finally, an important problem in traditional meta-analyses is publication bias especially reporting statistically significant results are more likely to be submitted for publication than those studies with non-significant results (Duval et al., 2000). This is because traditional meta-analysis are concerned with the effect size of the case control differences and that estimate can be affected by the non-publication of studies with non-significant (i.e. small effect size) results. This consideration does not apply in Activation Likelihood Estimation (ALE) meta-analysis as this method is concerned with the consistency of significant activations. Therefore, negative studies would not have contributed data to the ALE in any case whether they were published or not.

In general, a common method used to identify and estimate the extent of publication bias is the "funnel plot". This method plots the odd ratio on the horizontal axis and the sample size of each study in the vertical axis. Results from small studies will scatter widely at the bottom of the graph, with the spread narrowing among larger studies. In the absence of bias the plot will resemble a symmetrical inverted funnel. Conversely, if there is bias, funnel plots will often be skewed and asymmetrical. Another, more quantitative, method of assessing

publication bias consists in the Egger regression test which indicates the presence of a bias if the intercept of a regression line of effect size/standard error against 1/standard error significantly deviates from zero. If the resulting value yields a significant p-value, then this test indicates the presence of publication bias in the collection of studies (Egger et al., 1997).

3.7 Conclusion

In conclusion, we provided evidence for common and distinct neural correlates in BD, MDD and SZ in response to emotional faces. These results suggest (a) the need for more detailed examination of the relative contribution of medial temporal regions and particularly the interaction between amygdala and parahippocampus, (b) the contribution of cortical, thalamic and basal ganglia regions to the pathophysiology of mood disorders and suggest that examination of these cortico-thalamic-basal ganglia circuits may shed light to mechanisms differentiating BD from MDD, (c) that stimulus valence is an important modulator of activity within the neural networks underlying emotional processing in mood disorders, (d) that in SZ, affective modulation was reduced in the amygdala and additionally we show the same effect throughout the traditional face processing network, and (e) that facial affect identification deficits in SZ may arise from abnormalities in visual perception and represent another dimension of poor stimulus categorization.

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Table 3-1 Details of BD studies included in the meta-analysis (in alphabetical order)

Reference	Sample (M/W)	Clinical Diagnosis	Age Mean, years (standard deviation)	Design	Contrast
Almeida et al. (2010)	15 BD (remitted) (5/10) 15 BD (depressed) (1/14) 15 HI (3/12)	Euthymic and Depressed	BD (remitted): 33.2 (7.8) BD (depressed): 36.5 (11.8) MDD: 32.7 (9.8) HI: 32.6 (8)	Explicit facial affect labelling task, Event related	Sad > Neutral
Altshuler et al. (2008)	11 BD (5/6) 17 HI (9/8)	Depressed	BD: 32 (7.3) HI: 29.5 (6.6)	Explicit facial affect matching task, Block	Fearful and angry > Shapes
Blumberg et al. (2005)	17 BD (10/7) 17HI (7/10)	Unspecified	BD: 40 (12.3) HI: 33.2 (10.8)	Implicit facial affect recognition, Block	Happy > Fixation
Chen et al. (2006)	8 BD (depressed) (5/3) 8 BD (manic) (8/0) 8 HI (2/6)	Depressed and Manic	BD (depressed): 41.8 (12) BD (manic): 39 (13.4) HI: 38.7 (12.5)	Explicit facial affect labelling task and Implicit facial affect recognition, Event related	Fearful > Neutral (explicit) Happy > Neutral (explicit)
Foland et al. (2008)	9 BD (manic) (3/6) 9 HI (3/6)	Manic	BD: 34.6 (8.0) HI: 30.4 (7.6)	Explicit facial affect matching task, Block	Fearful and anger > Shapes
Foland-Ross et al. (2012)[1]	24 BD (15/9) 26 HI(15/11)	Euthymic	BD: 38.8 (12.8) HI : 37.9 (13.4)	Explicit facial affect matching task; Block	Label emotions> Shapes
Hassel et al. (2008)	19 BD (10/9) 24 HI (11/13)	Euthymic	BD: 32.47 HI: 27.78	Implicit facial affect recognition, Event related	Happy > Neutral
Hulvershorn et al. (2012)[1]	30 BD (depressed): 18/12) 30 BD (manic) (19/11) 15 BD (euthymic) (15/0) 30 HI (19/11)	Euthymic, Depressed, Manic	30 BD (depressed):35 (11) 30 BD (manic): 34 (11) 15 BD (euthymic):31 (11) 30 HI: 32 (10)	Explicit facial affect matching task; Block	Emotional faces > Shapes

Reference	Sample (M/W)	Clinical Diagnosis	Age Mean, years (standard deviation)	Design	Contrast
Jogia et al. (2008)[2]	12 BD (5/7) 12 HI (5/7)	Euthymic	BD: 42.1 (11.8) HI: 41.8 (10.9)	Explicit facial affect labelling task, Event related	Sad > Neutral Angry > Neutral
Killgore et al. (2008)	14 BD (11/3) 13 HI (12/1)	Manic	BD: 28.1 (11.2) HI: 25.5 (4.7)	Explicit face perception task, Block	Fearful > Fixation
Lawrence et al. (2004)[3]	20 BD 11 HI 60% Male	Euthymic	Overall mean 41 (11)	Implicit facial affect recognition task, Event related	Fearful > Neutral Happy > Neutral Sad > Neutral
Lennox et al. (2004)	10 BD (8/2) 12 HI (6/6)	Manic	BD: 37.3 (12.8) HI: 32.6 (10.7)	Explicit facial affect rating task, Event related	Sad > Neutral
Malhi et al. (2007)	10 BD (0/10) 10 HI (0/10)	Euthymic	BD: 33.5 (8.7) HI: 32.4 (6.4)	Explicit facial affect labeling task, Event related	Fearful > Neutral

BD=Bipolar Disorder; HI=Healthy Individuals; M=Men; W=Women.
1= results of these studies have been used only in one meta-analysis (Delvecchio et al., 2013); 2= only baseline data used; 3= study also included 9 Major Depressive Disorder patients.

Table 3-2 Details of SZ studies included in the meta-analysis (in alphabetical order)

Reference	Sample (M/W)	Age Mean, years (standard deviation)	Design	Contrast
Das et al. (2007)	14 SZ (14/0) 14 HI (14/0)	SZ:20.4 (3.3) HI: 23.1 (5.9)	Implicit facial affect viewing and Explicit facial affect labeling task, Block	Fearful > Neutral
Dowd et al. (2010)	32 SZ (20/12) 40 HI (26/14)	SZ:36.25 (10.85) HI: 36.8 (8.99)	Explicit valence rating task, Event Related	Fearful > Neutral
Fakra et al. (2008)	14 SZ (9/5) 14 HI (9/5)	SZ: 34.64 (5.96) HI: 37.29 (8.87)	Explicit facial affect matching task, Block	Fearful and angry > Shapes
Gur et al.(2002)	14 SZ (10/4) 14 HI (10/4)	SZ: 28.8 (8.9) HI:27.4 (7.3)	Implicit emotional valence discrimination, Block	Fearful, sad, angry, happy, disgust > fixation cross
Gur et al. (2007)	16 SZ (12/4) 17 HI(12/5)	SZ:30.1 (6.5) HI: 25 (3.9)	Explicit facial affect labeling task, Event related	Fearful > Neutral Angry > Neutral
Habel et al. (2010)	17 SZ 17 HI Sex unspecified	SZ: 34.4 (8.8) HI: 34.2 (7.7)	Explicit facial affect labeling task, Event related	Fearful > Baseline Angry > Baseline Happy > Baseline Sad > Baseline
Hall et al. (2008)	19 SZ (12/7) 24 HI (16/8)	SZ:37.7 (8.4) HI: 35.1 (9.7)	Implicit facial affect recognition, Block	Fearful > Neutral
Hempel et al. (2003)	9 SZ (4/5) 10 HI (6/4)	SZ: 28 HI:26	Explicit facial affect labeling task, Block	Fearful, sad, angry, happy, disgust and surprise > Baseline

Reference	Sample (M/W)	Age Mean, years (standard deviation)	Design	Contrast
Holt et al.(2006)	15 SZ (15/0) 16 HI (16/0)	SZ:47.7 (7.1) HI: 48.2 (9.6)	Implicit facial affect recognition, Block	Fearful > Neutral Happy > Neutral
Kosaka et al. (2002)	12 SZ (6/6) 12 HI(6/6)	SZ:26 (4.5) HI: 24.4 (2.4)	Emotional intensity judgment task, Block	Happy > Neutral
Lapage et al. (2011)	26 SZ (11/15) 26 HI (14/12)	SZ:31.8 (7.7) HI:28.3 (5.6)	Implicit facial emotion perception, Event related	Sad and happy > Neutral
Li et al. (2012)	12 SZ (6/6) 12 HI (6/6)	SZ: 29.8 (9.24)HI: 29.25 (7.24)	Explicit facial emotional valence discrimination, Event related	Happy >Neutral Fearful >Neutral
Michalopoulou et al. (2008)	11 SZ (9/2) 9 HI (5/4)	SZ:35 (9) HI: 32 (6)	Implicit facial affect recognition, Event related	Fearful > Neutral
Rauch et al.(2010)	12 SZ (7/5) 12 HI (9/3)	SZ: 27.7 (7.5) HI: 26.9 (6.1)	Implicit facial affect recognition, Block	Happy > Neutral Sad > Neutral
Reske et al. (2009)	18 SZ (10/8) 18 HI (10/8)	SZ:31.94 (6.41) HI: 31.94 (6.03)	Explicit facial affect labeling task, Event related	Sad > Neutral
Williams et al.(2007)	13 SZ (8/5) paranoid 14 SZ (9/5) (non-paranoid) 13 HI (8/5)	SZ:26.9 (9.1) paranoid SZ:27.8 (10.4) non-paranoid HI: 25.1 (8.1)	Implicit facial affect recognition, Block	Fearful > Neutral

HI=Healthy Individuals; M=Men; SZ= Schizophrenia; W=Women.

Table 3-3 Details of MDD studies included in the meta-analysis (in alphabetical order)

Reference	Sample (M/W)	Age Mean, years (standard deviation)	Design	Contrast
Fu et al. (2007)[1]	19 MDD (6/13) 19 HI (8/11)	MDD:43.2 HI:42.8	Implicit Facial Affect Processing, Event-Related	Happy > fixation
Gotlib et al. (2005)	18 MDD (5/13) 18 HI (5/13)	MDD: 35.2 HI:30.8	Implicit facial affect processing, Block	Happy > neutral Sad > neutral
Lawrence et al. (2004)	20 BD 9 MDD 11 HI 60% Male	Overall mean 41 (11)	Implicit facial affect processing , Event-Related	Fear>neutral Happy >neutral Sad > neutral
Lee et al. (2008)	21 MDD (3/18) 15 HI (2/13)	MDD= 46.8 (9.1) HI= 48.7 (3.5)	Explicit facial affect rating , Block	Sad> fixation
Norbury et al. (2009)	16 MDD (7/9) 21 HI (11/10)	MDD:36.2 HI:32.3	Explicit facial affect matching, Block	Fear vs Matching Shapes
Scheuerecker et al. (2010)	13 MDD (10/3) 15 HI (10/5)	MDD=37.9 (10.1) HI= 35.5 (10.9)	Implicit and explicit facial affect matching, Block	Sad and angry > shapes
Suslow et al. (2010)	30 MDD (17/13) 26 HI (12/14)	MDD: 38.8 (11.4) HI: 36.2 (13.4)	Implicit facial affect processing , Event-Related	Sad > neutral
Thomas et al. (2011)	30 rMDD (7/21) 37HI (12/23)	rMDD= 32.8 (10.4) HI= 31.7 (9.8)	Implicit facial affect processing , Block	Sad > neutral Fear > neutral

Reference	Sample (M/W)	Age Mean, years (standard deviation)	Design	Contrast
Townsend et al. (2010)	15 MDD (9/6) 15 HI (9/6)	MDD= 45.6 (11.2) HI= 44.8 (11.7)	Explicit facial affect matching, Block	Sad and angry > shapes
Van Wingen et al. (2010)	18 MDD (first episode) (7/11) 21 rMDD (4/17) 30 HI (13/17)	MDD (first episode): 33.3 (11.7) MDD (recovered): 34.5 (11.4) HI: 35 (12)	Explicit facial affect matching, Block	Fear and angry Emotion Labelling > shapes

HI=Healthy Individuals; MDD=Major Depressive Disorder; rMDD=remitted Major Depressive Disorder.
1=only baseline data used.

Table 3-4 Clinical Description of BD patients

Reference	Psychopathology Measures Mean, total score (standard deviation)	Duration of illness Mean, years (standard deviation)	Psychotropic Medication (number of patients)	Diagnostic Instrument	Comorbidities (number of patients with comorbid condition/total sample)	First or multiple episodes (standard deviation)	Symptomatic State[a]
Almeida et al. (2010)	YMRS scores BD (depressed): 21.53 (6.40) BD (remitted): 1.47 (1.13) MDD: 24.4 (6.1)	BD (depressed): 14.23 (9.82) BD (remitted): 14.67 (5.48) MDD: 13.67 (9.87)	Unspecified	SCID-P	9/30 BD substance abuse 3/15 MDD substance abuse	All patients had experienced at least two episodes of illness in the last 4 years	Depressed and remitted
Chen et al. (2006)	YMRS scores BD(manic): 24.13 (8.27) BD(depressed): 0.43 (0.53) HDRS scores BD (manic): 2 (2.98) BD (depressed): 18.38 (6.44)	Unspecified	Li (11), antiepileptics (9), antidepressants (2), typical (2) and atypical (3) antipsychotics	Unspecified	No comorbid axis I and II disorders	All patients had at least 2 previous episodes of mania and depression	Manic and depressed
Foland et al. (2008)	YMRS scores BD: 15.1 (3.7) HDRS scores BD: 9.1 (5.3)	14.8 (5.1)	Li (20), antiepileptics (6), atypical antipsychotics (1)	SCID-I	Excluded substance abuse No comorbid axis I disorders	Patients had experienced 4.2 (2.0) manic episodes on average	Manic
Foland-Ross et al. (2012)[1]	YMRS scores BD: 1.4 (1.9) HDRS scores BD: 4.6 (2.1)	21.2 (14.8)	Anticonvulsants (10), SGA (11), antidepressants (6)	SCID-I	No comorbid axis I disorders	Patients had experienced an average of 9.6 (10.7) depressive and 6.3 (8.7) manic episodes	Remitted

Reference	Psychopathology Measures Mean, total score (standard deviation)	Duration of illness Mean, years (standard deviation)	Psychotropic Medication (number of patients)	Diagnostic Instrument	Comorbidities (number of patients with comorbid condition/total sample)	First or multiple episodes	Symptomatic State[a]
Hassel et al. (2008)	YMRS scores BD:<10 HDRS scores BD: <7	BD: 10.6 (6.61)	Li (6), antiepilectics (7), typical (2) and atypical (12) antipsychotics, antidepressants (9) and benzodiazepines (4)	SCID-I	3/19 eating disorders 5/19 substance abuse 11/19 anxiety	Multi-episode unspecified	Remitted
Hulvershorn et al. (2012)[1]	YMRS scores BD (depressed): 3 (3) BD (manic): 16 (3) BD (euthymic): 2 (3) HDRS scores BD (depressed): 20 (4) BD (manic): 6 (3) BD (euthymic): 7 (4)	Unspecified	Unspecified	Diagnostic Interview for Genetic Studies and/or the Mini-International Neuropsychiatric Interview	Excluded substance abuse No comorbid axis I disorders	BD patients (depressed) have experienced a median of 15 (range: 2-186) depressive and 24 (1-310) manic episodes BD patients (manic) have experienced a median of 15 (2-102) depressive and 75 (3-447) manic episodes BD patients (euthymic) have experienced a median of 13 (0-100) depressive and 28 (1-122) manic episodes	Manic, depressive, remitted
Jogia et al. (2008)[2]	YMRS scores BD:<7 HDRS scores BD:<14	Unspecified	antiepilectics (12)	SCID-I	Excluded substance abuse No comorbid axis I and II disorders	The patients had an average of 10.1 (6.5) episodes	Remitted

Reference	Psychopathology Measures Mean, total score (standard deviation)	Duration of illness Mean, years (standard deviation)	Psychotropic Medication (number of patients)	Diagnostic Instrument	Comorbidities (number of patients with comorbid condition/total sample)	First or multiple episodes	Symptomatic State[a]
Lawrence et al. (2004)[3]	BDI Scores BD: 15.3 (9.2) MDD: 31.8 (11.8)	BD: 15.4 (13.4) MDD: 8 (5)	Li (3), antiepilectics (7), atypical antipsychotics (5), antidepressants (5)	Unspecified	Excluded substance abuse No comorbid axis I and II disorders	Multi-episode unspecified	Depressed and remitted
Malhi et al. (2007)	YMRS scores BD: <6 HDRS scores BD:< 6	BD: 12.0 (7.7)	Li (3), antiepilectics (5)	SCID-P	Excluded substance abuse No comorbid axis I and II disorders	Patients had experienced an average of 10.4 (8.7) depressive and 4.7 (3.4) manic episodes	Remitted
Altschuler et al. (2008)	HRSD scores BD (depressed):20.8 (3.3) HDRS (7 items extension) score BD (depressed): 31.27 (4.76) YMRS score BD:2.9 (1.9)	Unspecified	medication free (2), antiepilectics (7), Li (1), atypical antipsychotics (2), antidepressants (3)	SCID-I	Excluded substance abuse No comorbid axis I disorder	Unspecified	Depressed
Killgore et al. (2008)	YMRS scores BD: 14.3 (8.9) HDRS scores BD: 15.6 (9.9)	BD: < 1 year	Li or antiepilectics (5) atypical antipsychotics (12), benzodiazepines (4), antidepressants(1)	SCID-P	Unspecified	First episode bipolar patients	Remitted

Reference	Psychopathology Measures Mean, total score (standard deviation)	Duration of illness Mean, years (standard deviation)	Psychotropic Medication (number of patients)	Diagnostic Instrument	Comorbidities (number of patients with comorbid condition/total sample)	First or multiple episodes	Symptomatic State[a]
Blumberg et al. (2005)	HDRS ARSM Unspecified scores	Unspecified	medication free (5), antiepilectics (8), Li (4), antidepressants (3), atypical antipsychotics (1)	Unspecified	2/ 5 (unmedicated) 9/12 (medicated) with alcohol dependence	Unspecified	Remitted, manic and depressed

ASRM=Altman Self-Rating Mania Scale; BD=Bipolar Disorder; BDI=Beck Depression Inventory; HDRS=Hamilton Depression Rating Scale; Li= Lithium; SCID=Structured Clinical Interview for DSM-IV; YMRS=Young Mania Rating Scale.

[a]We used the description of symptomatic state as in the original articles; 1= results of these studies have been used only in one meta-analysis (Delvecchio et al., 2013); 2= only baseline data used; 3= study also included 9 Major Depressive Disorder patients.

Table 3-5 Clinical Description of SZ patients

Reference	Psychopathology Measures Mean, total score (standard deviation)	Number of Episodes Duration of Illness Mean, years (standard deviation)	Psychotropic medication (number of patients)	Comorbidities	Symptomatic State[a]
Das et al. (2007)	PANSS Positive: 16.07 (7.24) PANSS Negative: 21.14 (7.9)	First episode psychosis; Duration of illness: 1.21 (1.2) years	SGA (9)	No substance abuse	Remitted
Dowd et al. (2010)	SAPS: 1.83 (.93) SANS: 1.81 (1.37)	Unspecified; Duration of illness: 17.73 (11.25) years	SGA and FGA (number unspecified)	No substance abuse, No comorbid axis I disorders	Remitted
Fakra et al. (2008)	PANNS Positive: 24.71 PANSS Negative: 13.14 (5.46)	Unspecified	Antipsychotics (unspecified)	No substance abuse	Remitted
Gur et al. (2002)	SAPS: 0.5 (5) HDRS: 6.0 (4.7)	Unspecified	FGA (1), SGA (11)	No comorbid axis I and II disorders	Remitted
Gur et al. (2007)	SAPS: 1.4 (0.6) SANS: 1.3 (0.9)	Unspecified; Duration of illness: 9.6 (7.1) years	FGA (2), SGA (11), combination of FGA and SGA (2)	No comorbid axis I and II disorders	Remitted
Habel et al. (2010)	PANSS Positive:18.0 (7.3) PANSS Negative:19.9 (8.8) PANSS General:76.5 (26.3)	Unspecified	FGA (2), SGA (12), combination of FGA and SGA (2), unmedicated (1)	No comorbid axis I and II disorders	Remitted
Hall et al. (2008)	PANSS Positive: 12.3 (4.5)	Unspecified	FGA (3) and SGA (16)	No substance abuse, No comorbid axis I and II disorders	Remitted

Reference	Psychopathology Measures Mean, total score (standard deviation)	Number of Episodes Duration of Illness Mean, years (standard deviation)	Psychotropic medication (number of patients)	Comorbidities	Symptomatic State[a]
Hempel et al. (2003)	PANSS Positive:13 PANSS Negative:13 PANSS General: 18	Unspecified; Duration of illness: 13 months	SGA (9)	No substance abuse	Remitted
Holt et al. (2006)	PANSS total: 59.8 (10.3)	Unspecified; Duration of illness: 21.6 (9.6) years	Unspecified	No substance abuse	Remitted
Kosaka et al. (2002)	PANSS Positive: 11.3 (4.6) PANSS Negative: 16.3 (4.5) PANSS General: 28.8 (7.3)	Unspecified; Duration of illness: 3.8 (3.5) years	Antipsychotics (unspecified)	Unspecified	Remitted
Lapage et al. (2011)	SAPS: 9.6 (10.2) SANS: 15.6 (9.1)	Unspecified; Duration of illness: 8.5 (6.5)	SGA (18), combination of SGA and FGA (6), anticholinergic medication (1), combination of SGA /FGA and antidepressants (7)	No comorbid axis I Disorders	Remitted
Li et al. (2012)	PANSS Positive 16.08 (5.66) PANSS Negative: 13.41 (8.89)PANSS total: 28.42 (13.82)	Unspecified; Duration of illness: 5.42 (3.75) years	Antipsychotics (unspecified)	No comorbid axis I and II disorders	Remitted
Michalopoulou et al. (2008)	PANSS positive: 16 (6.72) PANSS negative: 13.91 (5.54) PANSS total: 58.91 (17.72)	Unspecified; Duration of illness: 12 (9) years	FGA (8), SGA (3)	No substance abuse	Remitted

Reference	Psychopathology Measures Mean, total score (standard deviation)	Number of Episodes Duration of Illness Mean, years (standard deviation)	Psychotropic medication (number of patients)	Comorbidities	Symptomatic State[a]
Rauch et al.(2010)	PANSS Positive: 14.4 (3.1) PANSS Negative: 18.9 (5.2) PANSS General: 34.9 (5.9)	Unspecified	SGA (9), combination of FGA and SGA (3)	No substance abuse, No comorbid axis I disorders	Remitted
Reske et al. (2009)	PANSS Positive: 8 (1.14) PANSS Negative: 13.61 (4.47) PANSS total: 23.11 (3.94)	First episode psychosis; Unspecified	FGA (9), SGA (9)	No comorbid axis I disorders	Remitted
Williams et al.(2007)	PANSS delusions: 2.1 (1.1); excitement: 1.6 (0.8)	Unspecified; Duration of illness: 5.6 (4.6) years	SGA	No substance abuse	Remitted

FGA=Frst-Generation Antipsychotic; HDRS=Hamilton Depression Rating Scale; HI=Healthy Individuals; PANSS=Positive and Negative Syndrome Scale; SGA=Second-Generation Antipsychotic; SANS=Scale for the Assessment of Negative Symptoms; SAPS=Scale for the Assessment of Positive Symptoms; SZ=Schizophrenia.

[a]We used the description of symptomatic state as in the original articles.

Table 3-6 Clinical Description of MDD patients

Reference	Psychopathology Measures Mean, total score (standard deviation)	Duration of illness Mean, years (standard deviation)	Psychotropic Medication (number of patients)	Diagnostic Instrument	Comorbidities (number of patients with comorbid condition/total sample)	First or multiple episodes	Symptomatic State[a]
Fu et al. (2007)[1]	HDRS score MDD: 21.1 (2.3)	Unspecified	Medication free at baseline	SCID	No comorbid axis I and II disorders	Multi-episode unspecified	Depressed
Gotlib et al. (2005)	BDI score MDD: 24.6 (8.3)	Unspecified	Antidepressants (8)	SCID	No comorbid axis I and II disorders	Multi-episode unspecified	Depressed
Lee et al. (2008)	HDRS score MDD: 22.2 (4)	MDD: 14.9 (8.8)	antidepressants (10)	SCID	No comorbid axis I and II disorders	Mean depressive episodes: 1.9 (0.8)	Depressed
Norbury et al. (2009)	BDI score MDD: 3.5 (3.7)	Unspecified	medication free	Unspecified	1/16 anxiety disorders 2/16 alcohol misuse	Patients with at least 2 previous episode of depression	Remitted
Scheuerecker et al. (2010)	HDRS score MDD: 20.5 (4.7)	MDD: 52.3 (71.5)	medication free	SCID	No comorbid axis I and II disorders	8 patients with first episodes and 5 with recurrent episodes Mean depressive episodes: 1.4 (0.6)	Depressed
Suslow et al. (2010)	HDRS score MDD: 24.8 (4.9)	MDD: 6 (6.2)	antidepressants (30)	SCID	13/30 anxiety disorders 3/30 dysthymia 1/30 pain disorder	Mean depressive episodes: 2.7 (2)	Depressed

Reference	Psychopathology Measures Mean, total score (standard deviation)	Duration of illness Mean, years (standard deviation)	Psychotropic Medication (number of patients)	Diagnostic Instrument	Comorbidities (number of patients with comorbid condition/total sample)	First or multiple episodes	Symptomatic State[a]
Thomas et al. (2010)	MADRS score MDD: 2.3 (3.2)	Unspecified	antidepressants (3)	SCID	No comorbid axis I and II disorders	Unspecified	Remitted
Townsend et al. (2010)	HDRS score MDD: 20.1 (4.9)	MDD: 14.7 (13.3)	medication free	SCID	No comorbid axis I and II disorders	Median depressive episodes: 3	Depressed
Van Wingen et al. (2010)	HDRS score MDD (depressed): 21.8 (4.2) MDD (remitted): 3.3 (2)	Unspecified	medication free	SCID	No comorbid axis I and II disorders	18 with first MDE and 18 recovered from a first MDE	Depressed and Remitted

BDI=Beck Depression Inventory; HDRS=Hamilton Depression Rating Scale; MDD=Major Depressive Disorder; MDE=Major Depressive Episode; SCID=Structured Clinical Interview for DSM-IV. [a]We used the description of symptomatic state as in the original articles; 1= only baseline data used.

Table 3-7 Results from the global Activation Likelihood Estimation (ALE) analyses of facial affect processing in Bipolar Disorder (p< 0.05 False Discovery Rate corrected)

				Centre of maximum ALE				
Brain region	**Gyrus**	**BA**	**Laterality**	**X**	**Y**	**Z**	**Volume (mm^3)**	**Maximum ALE value**
Bipolar Disorder > Healthy individuals								
Limbic	Amygdala	-	Right	24	-4	-16	392	0.01
			Left	-18	-4	-16	1048	0.01
	Parahippocampal	28	Left	-18	-18	8	1048	0.01
Putamen	-	-	Left	-26	-6	-8	1048	0.01
Thalamus	Pulvinar	-	Left	-6	-26	4	368	0.01
Bipolar Disorder < Healthy individuals								
Frontal	Inferior Frontal	47	Left	-34	26	-8	480	0.01
		47	Right	36	30	-8	872	0.01

L =left; R=right; x=sagittal, y=coronal, z=axial coordinates according to Talairach and Tournoux.

Table 3-8 Results from the valence Activation Likelihood Estimation (ALE) sub-analyses of facial affect processing in Bipolar Disorder (p< 0.05 False Discovery Rate corrected)

				Centre of maximum ALE				
Brain region	**Gyrus**	**BA**	**Laterality**	**X**	**Y**	**z**	**Volume (mm^3)**	**Maximum ALE value**
Fear								
Bipolar Disorder > Healthy individuals								
Limbic	Parahippocampal	28	Left	-18	-12	-12	584	0.01
Putamen	-	-	Left	-26	-6	-8	584	0.008
Thalamus	Pulvinar	-	Left	-6	-26	4	346	0.01
Bipolar Disorder < Healthy individuals								
Frontal	Inferior Frontal	47	Left	-34	24	-8	480	0.01
		47	Right	44	22	-2	1080	0.01
		47	Right	52	12	28	408	0.01
Limbic	Anterior Cingulate	32	Left	-8	34	12	424	0.01
Happy								
Bipolar Disorder > Healthy individuals								
Limbic	Parahippocampal	34	Left	-26	6	-16	80	0.008
Caudate	-	-	Left	-18	20	14	80	0.009
	-	-	Right	14	10	16	96	0.009
	-	-	Right	18	24	-6	80	0.008
Bipolar Disorder < Healthy individuals								
Limbic	Anterior Cingulate	32	Right	20	14	36	80	0.009

L =left; R=right; x=sagittal, y=coronal, z=axial coordinates according to Talairach and Tournoux.

Table 3-9 Results from the global and valance Activation Likelihood Estimation (ALE) analyses of facial affect processing in Major Depressive Disorder and the direct comparison with Bipolar Disorder (p< 0.05 False Discovery Rate corrected)

				Centre of maximum ALE				
Brain region	**Gyrus**	**BA**	**Laterality**	**X**	**Y**	**z**	**Volume (mm^3)**	**Maximum ALE value**
Global Activation Likelihood Estimation (ALE) analysis								
Major depressive disorder > healthy individuals								
Limbic	Amygdala	-	Right	28	0	-16	232	0.01
			Left	30	-4	-20		
Major depressive disorder < Healthy individuals								
Basal ganglia	Putamen	-	Right	28	-1	0	400	0.01
	Caudate	-	Left	-36	-14	-10	256	0.01
Bipolar Disorder > Major Depressive Disorder								
Limbic	Amygdala	-	Left	-18	-4	-14	2984	0.01
				-18	-30	-8		0.008
				-24	6	-16		0.008
	Anterior cingulated	32	Right	8	24	24	376	0.007
			Right	6	30	28		
Thalamus	Pulvinar	-	Left	-6	-26	6	584	0.01
Bipolar Disorder < Major Depressive Disorder								
Limbic	Anterior cingulated	24	Left	-20	-18	46	416	0.007
Valence Activation Likelihood Estimation (ALE)								
Fear								
Major Depressive Disorder > Healthy individuals								
No suprathreshold clusters								
Major Depressive Disorder < Healthy individuals								
Frontal	Precentral	6	Left	-58	0	12	96	0.009
Happy								
Major Depressive Disorder > Healthy individuals								
No suprathreshold clusters								
Major Depressive Disorder < Healthy individuals								
Thalamus	Pulvinar		Right	6	-28	4	368	0.01

L=left; R=right; x=sagittal, y=coronal, z=axial coordinates according to Talairach and Tournoux.

Table 3-10 Results from the global Activation Likelihood Estimation (ALE) analyses of facial affect processing in Schizophrenia and the direct comparison with Bipolar Disorder (p< 0.05 False Discovery Rate corrected)

				Centre of maximum ALE				
Brain region	**Gyrus**	**BA**	**Laterality**	**X**	**Y**	**Z**	**Volume (mm^3)**	**Maximum ALE value**
Schizophrenia > Healthy individuals								
Occipital	Cuneus	18	Right	10	-88	20	416	0.01
Schizophrenia < Healthy individuals								
Frontal	Medial Frontal	9	Left	-8	46	22	360	0.01
	Precentral	6	Left	-34	2	32	856	0.01
Limbic	Parahippocampal	28	Left	-18	-2	-14	1104	0.01
	Anterior cingulated	32	Left	-12	26	22	408	0.01
	Amygdala	-	Right	20	-10	-8	384	0.01
	Insula	13	Right	40	-18	2	376	0.01
Occipitotemporal	Fusiform	37	Right	38	-48	12	592	0.01
Occipital	Inferior Occipital	19	Right	12	-92	-4	328	0.01
Basal ganglia	Caudate	-	Right	8	4	-4	408	0.01
Thalamus	Medial Dorsal	-	Right	10	10	18	1016	0.01
Bipolar Disorder > Schizophrenia								
Thalamus	Pulvinar		Left	-5	-26	6	336	1.9
Schizophrenia > Bipolar Disorder								
Occipital	Cuneus	18	Left	-6	-92	18	1144	1.7
		18	Right	10	-88	20	416	1.7

L=left; R=right; x=sagittal, y=coronal, z=axial coordinates according to Talairach and Tournoux

Figure 3-1 Activation Likelihood Estimation (ALE) maps representing regional activity consistently associated with Bipolar Disorder. Clusters of relative overactivation or underactivation are shown in red and blue respectively; numbers represent axial (z) coordinates of each slice in Talairach space; p< 0.05 False Discovery Rate corrected for multiple comparisons. Top row: statistical map of significant ALE clusters for the comparison of BD patients to healthy individuals (HC). Middle row: statistical map of significant ALE clusters for the comparison of BD patients to healthy individuals (HC) during processing of happy stimuli. Bottom row: statistical maps of significant ALE clusters associated with the contrast of BD patients to healthy individuals (HC) during processing of fearful stimuli.

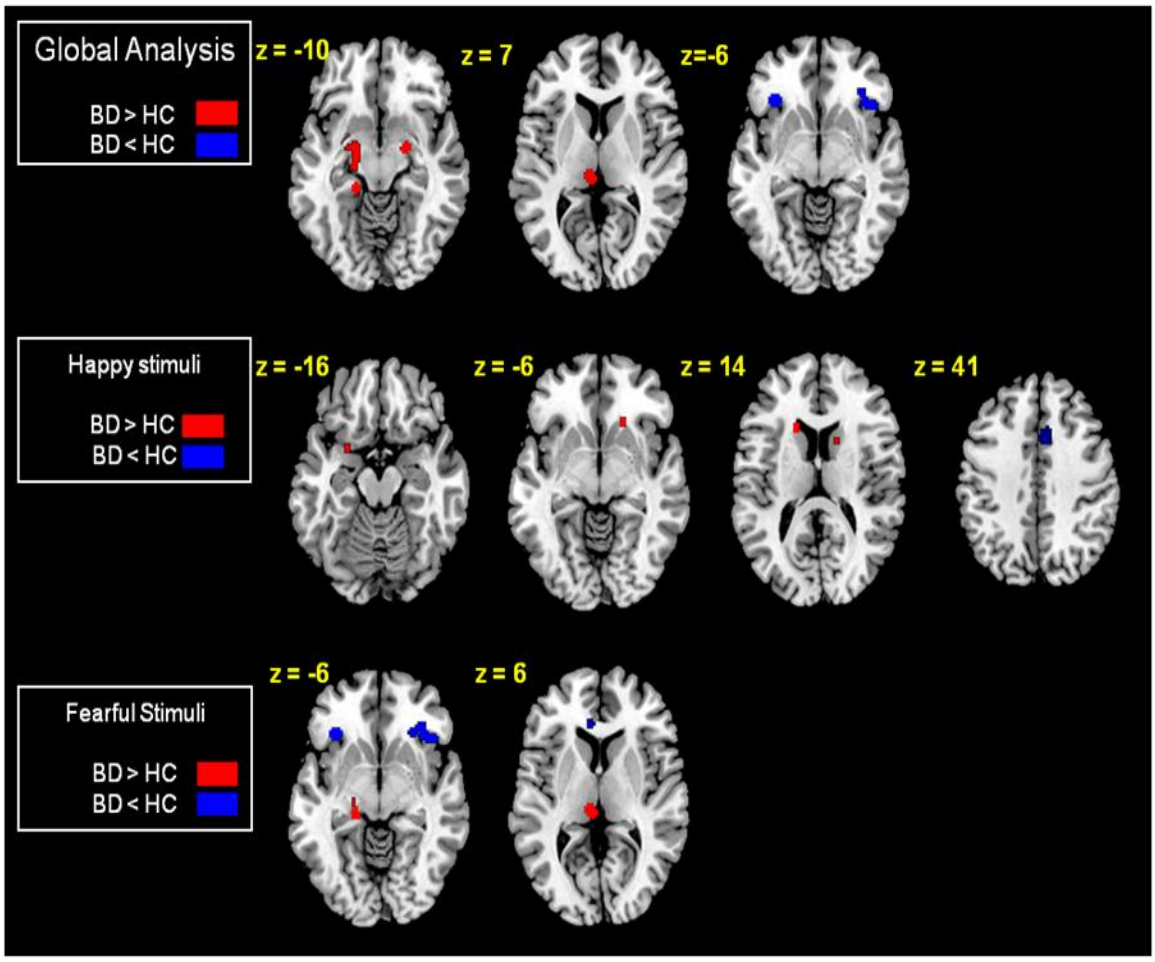


Figure 3-2 Activation Likelihood Estimation (ALE) maps representing regional activity consistently associated with Major Depressive Disorder (MDD). Clusters of relative overactivation or underactivation are shown in red and blue respectively; numbers represent axial (z) coordinates of each slice in Talairach space; p< 0.05 False Discovery Rate corrected for multiple comparisons. Top row: statistical map of significant ALE clusters for the comparison of MDD patients to healthy healthy individuals (HC). Bottom row: statistical maps of significant ALE clusters associated with the contrast of BD and MDD.

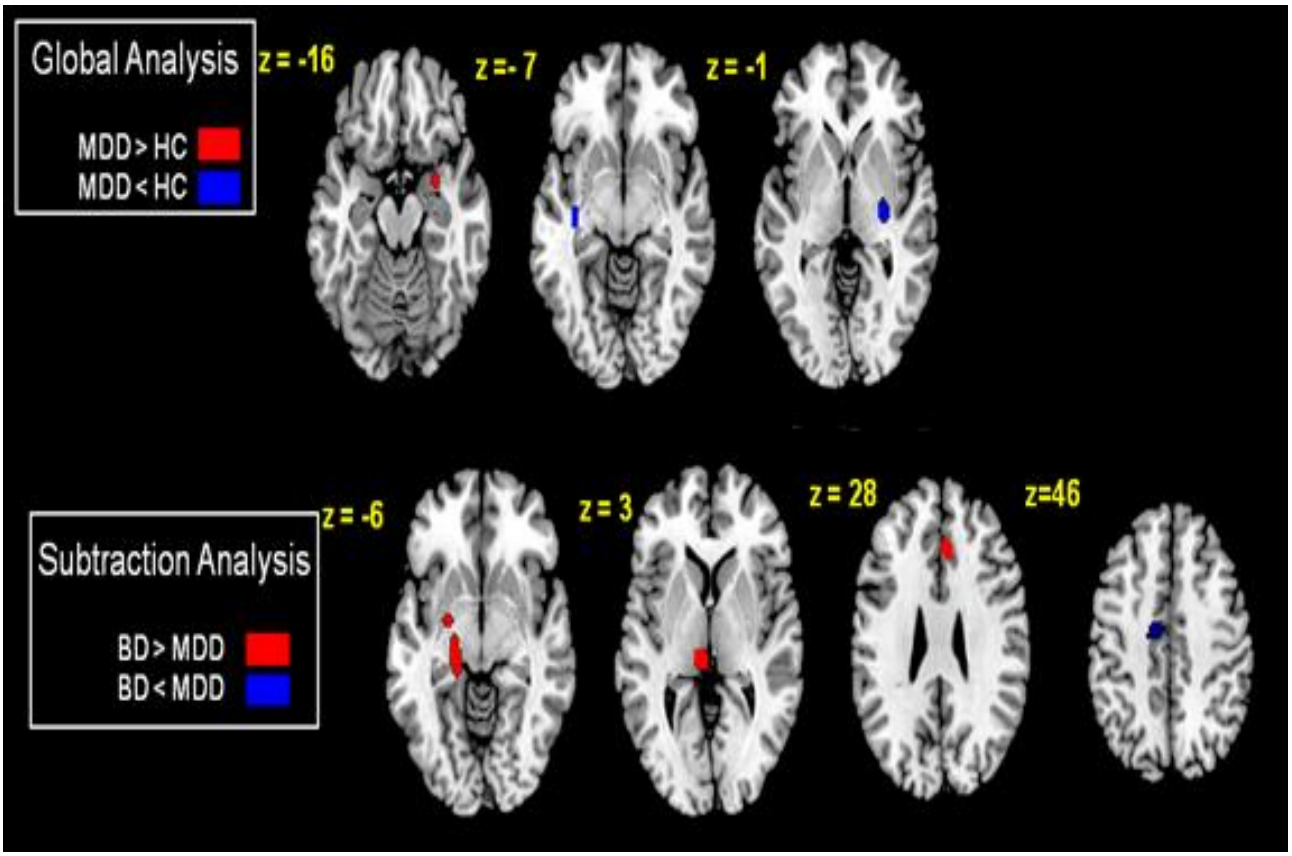
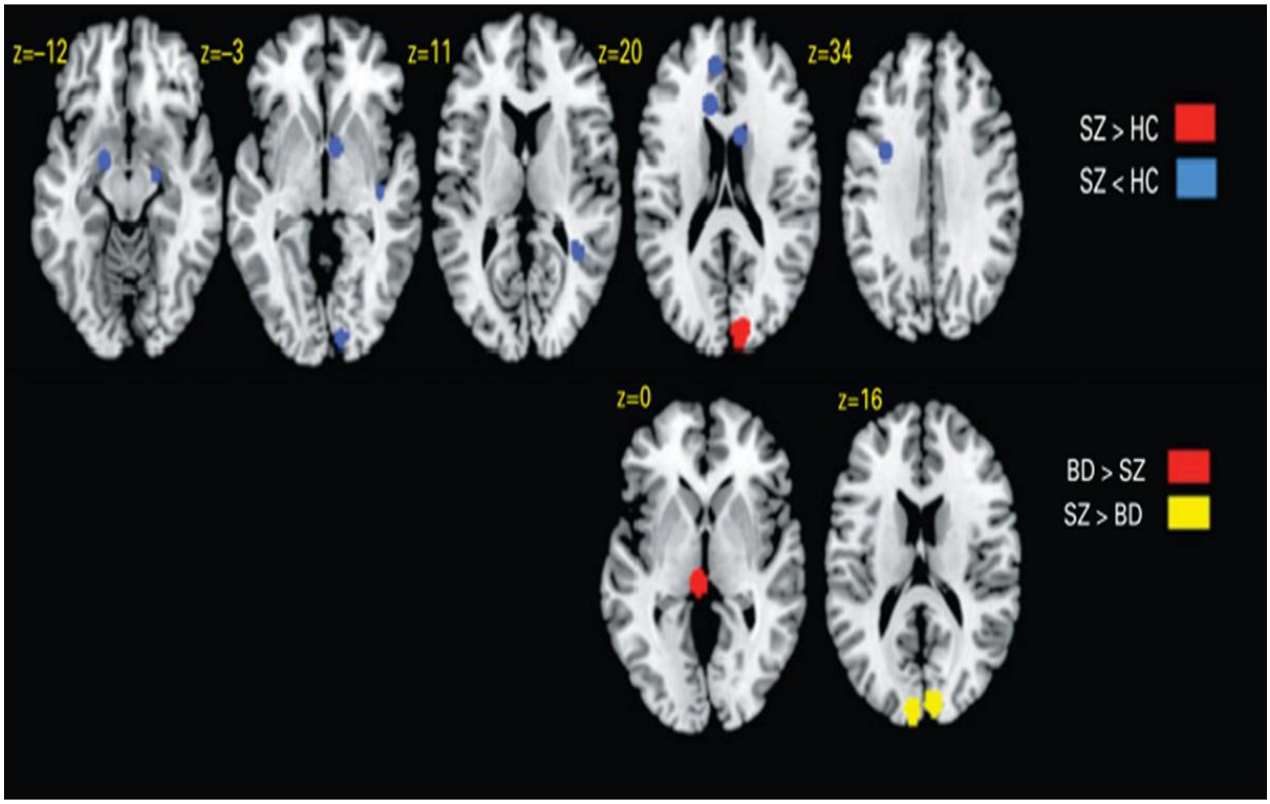
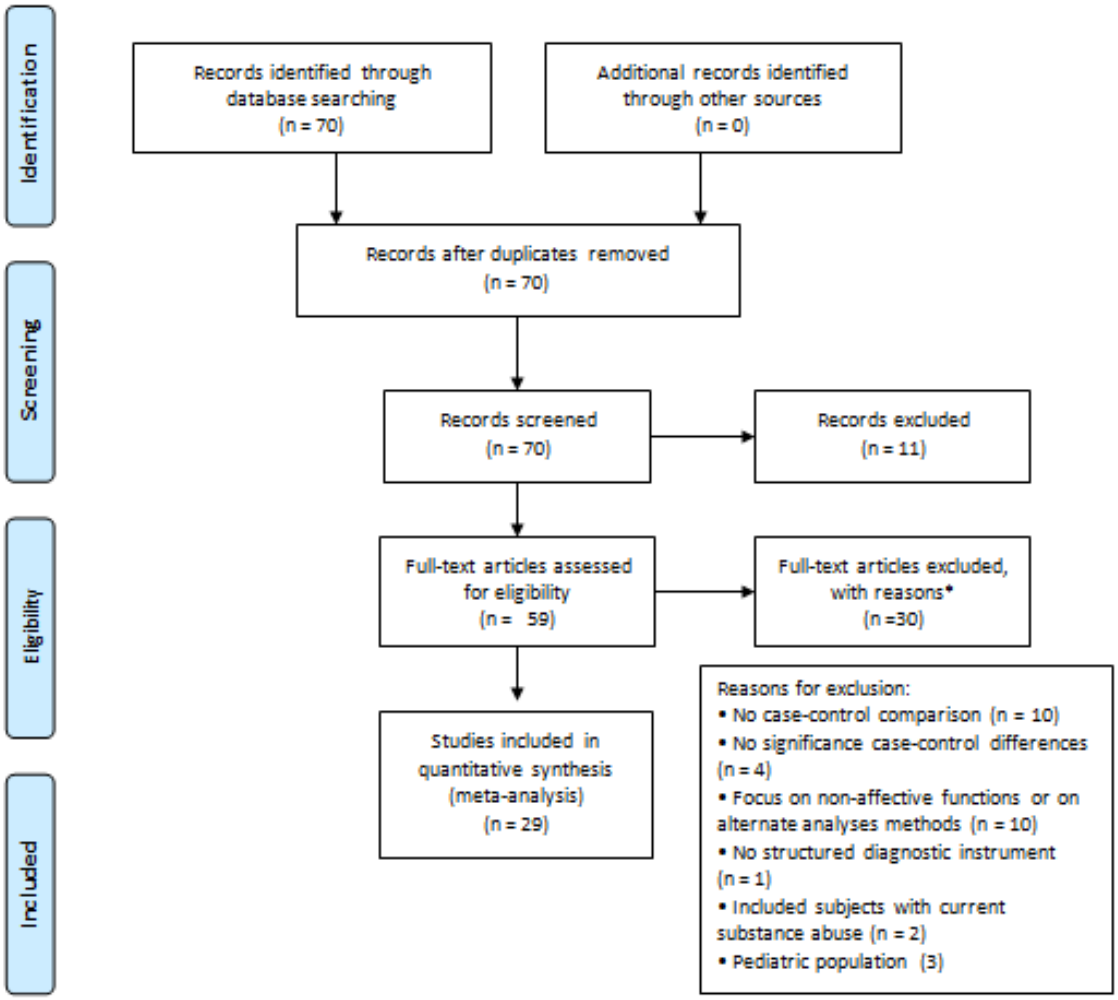


Figure 3-3 Activation likelihood estimation (ALE) maps representing regional activity consistently associated with schizophrenia (SZ). Top row: SZ patient compared to healthy healthy individuals (HC). Bottom row: SZ patients compared to BD patients. $p<0.05$ false discovery rate corrected.



Supplementary Figure 3-S1 Flowchart of study selection



4. Genetics of Bipolar Disorder

4.1 Heritability of Bipolar Disorder

Heritability is the proportion of trait variation that is accounted for by genetic factors, as opposed to trait variation due to environmental factors. Twin and family studies for bipolar disorder (BD) have placed heritability from 0.6 to 0.85 (McGuffin et al., 2003; Craddock and Sklar, 2009). To date, the single most significant predictor of BD expression remains genetic proximity to affected individuals; with first-degree relatives of BD patients showing a 10-fold increase in their risk to develop BD (Smoller et al., 2003). However, the majority of first-degree relatives (around 60%) of BD patients do not develop any psychiatric disorder (Mowbray et al., 2006). Recent meta-analyses of cognitive functioning in unaffected first-degree relatives of BD patients (RELs) have shown similar deficits to those observed in patients with BD, although milder, especially in verbal memory and some aspects of executive functions, including response inhibition, sustained attention and speed processing (Arts et al., 2008; Balanza-Martinez et al., 2008; Bora et al., 2009a). Moreover, a twin study by Christensen et al. (2006) compared several cognitive domains including attention, visuospatial abilities, language, learning and problem solving in a sample of 21 pairs of twins discordant for BD and 88 pairs of healthy twins. The results showed that also the unaffected twin pairs discordant for BD performed worse in comparison to healthy twins in working memory and declarative memory measures. Lastly, a recent study found that processing speed, long-term memory, verbal fluency, working memory, sustained attention and inhibitory controls are significantly heritable and that most of these measures are impaired in BD (Fears et al., 2014).

4.2 FMRI studies in RELs of BD patients

With regard to fMRI studies, the direct comparison between RELs and healthy individuals suggested that RELs showed impairment in several cortical and subcortical regions while processing of cognitive and emotional tasks.

During an easy and hard condition of a verbal fluency task, Allin et al. (2010) showed that RELs demonstrated hyperactivation in the retrosplenial cortex and the precuneus and

reduced activation in the frontotemporal areas compared to healthy individuals. A recent study by Sepede et al. (2012) showed that during a sustained attention task RELs shared a common pattern of activation with BD patients with increased activation in the posterior middle cingulate cortex and insula compared to healthy individuals. Linke et al. (2012) reported that during a reversal learning task, including a reward, punishment and reversal condition, RELs had increased activation in the medial orbitofrontal cortex (OFC) in all three conditions compared to healthy individuals. In the same study RELs also showed increased activation in the amygdala in response to reward compared to healthy individuals and BD patients. Two fMRI studies explored the neural activation (Pompei et al., 2011b) and functional connectivity (Pompei et al., 2011a) in BD patients, RELs and healthy individuals while performing the Stroop task. Together their findings suggested that RELs had decreased activation in the inferior and superior parietal lobule as well as reduced ventrolateral prefrontal cortex (VLPFC) connectivity with the ventral anterior cingulate cortex (ACC) and insula compared to healthy individuals. Two fMRI studies by Thermenos et al. (2010) and Drapier et al. (2008) explored brain activation in BD patients, RELs and healthy individuals while performing the 2-back version of the N-back task- a working memory task. The authors suggested that RELs showed increased activation in the insula, OFC, superior parietal lobule and postcentral gyrus (Thermenos et al., 2010) as well as in the frontal poles bilaterally (Drapier et al., 2008) compared to healthy individuals. Finally, in a facial emotional task, Surguladze et al. (2010) found increased medial PFC and putamen activation to fearful or happy faces in RELs compared to healthy individuals. In an additional region of interest analysis, the authors also reported an increased activation of the amygdala in RELs compared to healthy individuals in response to happy faces. Details of the original studies that included RELs in their samples have been reported in Table 4-1.

Table 4-1 FMRI studies in unaffected first-degree relatives of patients with BD

Reference	Sample (M/W)	Age Mean, years (standard deviation)	Design	Contrast	Results
Allin et al. (2010)	18 euthymic BD (7/11) 19 RELs (11/8) 19 HI (10/9)	BD: 39.2 (11.5) RELs: 40.5 (13.9) HI: 39.9 (11.0)	Verbal Fluency task, Event related	Easy and hard verbal fluency condition	RELs > HI, easy condition: increased activation in the retrosplenial/PCC and precuneus and reduced activation in the left frontotemporal areas RELs > HI, hard condition: increased retro-splenial/posterior cingulate cortex, and reduced activation in the medial frontal cortex
Drapier et al. (2008)	20 euthymic BD (9/11) 20 RELs (12/8) 20 HI (10/10)	BD: 42.7 (10.4) RELs: 43 (13.8) HI:41.9 (11.6)	N-back, Block	1-,2-,3- back vs 0-back	RELs > HI, 2-back vs 0-back: increased activation in the right and left frontal pole
Linke et al. (2012)	19 euthymic BD (8/11) 22 RELs (11/11) 22 HI matched to RELs (8/11) 22 HI matched to BD (11/11)	BD: 45 (10) RELs: 28 (11) HI (RELs): 45 (10) HI(BD): 28 (10)	Reversal Learning Task, Event related	Win vs baseline (reward) lose/no shift vs baseline (punishment) Lose/shift vs baseline (reversal)	RELs > HI, Reward, Punishment, Reversal: increased activation in the medial orbitofrontal cortex RELs >HI, Reward, Reversal: Increased amygdala
Pompei et al. (2011a)[1]	39 euthymic BD (19/20) 25 RELs (13/12) 48 HI (25/23)	BD: 39.43 (11.5) RELs: 35.0 (13.67) HI:36.33 (12.8)	PPI analyses, Stroop Colour Word Test, Block	Incongruent vs neutral condition	RELs < HI: reduced VLPFC connectivity with the vACC RELS < HI: reduced VLPFC connectivity with the insula VLPFC

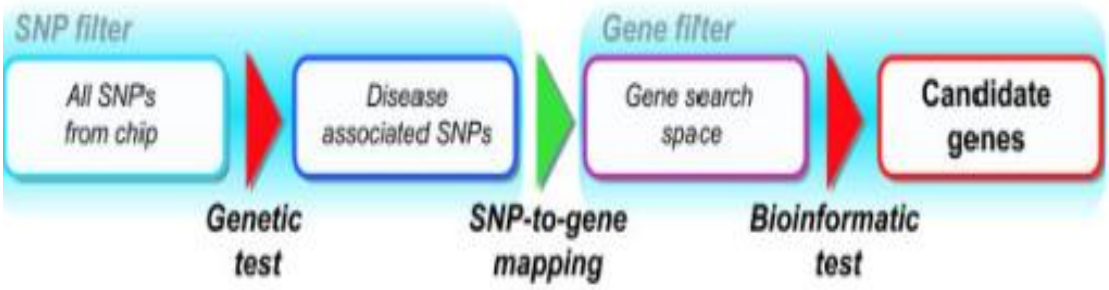
Reference	Sample (M/W)	Age Mean, years (standard deviation)	Design	Contrast	Results
Pompei et al. (2011b)[1]	39 euthymic BD (19/20) 25 RELs (13/12) 48 HI (25/23)	BD: 39.43 (11.5) RELs: 35.0 (13.67) HI:36.33 (12.8)	Stroop Colour Word Test, Block	Incongruent vs neutral condition	RELs < HI: reduced activation in the the superior and inferior parietal lobule
Sepede et al. (2012)	24 euthymic BD (10/14) 22 RELs (7/15) 24 HI (8/16)	BD: 34.8 (8.0) RELs: 31.5 (7.3) HI:32.5 (6.2)	Continuous Performance Task, Event related	Correct and Incorrect target condition vs correct response to non-targets	RELs > HI, Incorrect target condition: Increased activation in the posterior middle cingulate cortex and left insula
Surguladze et al. (2010)	20 euthymic BD (9/11) 20 RELs (12/8) 20 HI (10/10)	BD: 42.7 (10.4) RELs: 43 (13.8) HI:41.9 (11.6)	Facial emotion processing task, Event related	Moderate or intensive expressions of fear vs neutral Moderate or intensive expressions of happiness vs neutral	RELs > HI, Happiness or Fear: increased activation in the medial prefrontal cortex and putamen. Region of interest analysis in the amygdala showed also increased activation in the amygdala in RELs in response to intensively happy faces
Thermenos et al. (2010)	19 euthymic BD (11/8) 18 RELs (8/10) 19 HI (9/10)	BD: 41.1 (3.1) RELs: 36.3 (2.6) HI:39.2 (2.7)	N-back, Block	2-back vs 0-back	RELs > HI: increased activation in the insula, OFC and the right superior parietal lobule and the postcentral gyrus

BD= Bipolar Disorder; HI= Healthy Individuals;M=Males; PPI= Psychophysiological interaction; RELs= Unaffected first-degree relatives of BD patients;W=Women.
[1] The study included also a sample of Relatives of Major Depressive Disorder patients.

4.3 Genome-Wide Association Studies in Bipolar disorder

After the human genome was first sequenced the genetics field has produced huge technological strides towards estimating heredity (Lander et al., 2001). The most common genetic marker is the single nucleotide polymorphism (SNP), a single subunit change in the DNA sequence. SNPs are located on a DNA chip, which is used to create a genetic profile for each individual. In turn DNA chips are used in genome-wide associations studies (GWAS) that estimate the heritability of a disorder throughout the genome by comparing healthy controls with patients, in a hypothesis-free way. The first disease upon which the GWAS technique was used was the age-related macular degeneration in 2005 (Klein et al., 2005), and since then many genes have been implicated in the disease (Fritsche et al., 2013). A schematic representation of GWAS analysis methodology is reported in Figure 4-1.

Figure 4-1 Schematic representation of GWAS analysis methodology.



Ballouz et al., BMC Genet. (2011)

With regard to BD, several independent GWAS studies reported numerous SNPs associated with BD, with the strongest confirmation of two loci, the CACNA1C and the ANK3, in both European/American (Baum et al., 2008; Dedman et al., 2012; Sklar et al., 2008; Ferreira et al., 2008; Scott et al., 2009; Sklar et al., 2011; Green et al., 2013; Mühleisen et al., 2014; Schulze et al., 2009) and Asian (Chen et al., 2013; Lee et al., 2011a; Takata et al., 2011) ancestry.

Particularly for the CACNA1C gene, the majority of GWAS studies found one SNP, the rs1006737, to be strongly associated with BD (Sklar et al., 2008, 2011; Ferreira et al., 2008; Green et al., 2013). Moreover, these GWAS studies also identified another candidate gene with the strongest evidence for association with BD: the ANK3 gene for the imputed SNP

rs10994336. Also in this case, the association between BD and ANK3 rs10994336 gene has been replicated by other research groups in independent samples from European (Scott et al., 2009; Schulze et al., 2009; Mühleisen et al., 2014) and Asian (Chen et al., 2011; Lee et al., 2011) ancestry. Specifically, in the most recent and largest GWAS study of 9,747 BD patients and 14,278 healthy individuals, Mühleisen et al. (2014) found 56 genome-wide significant SNPs in five chromosomal regions, including risk loci within the ANK3 rs10994336. Finally, further evidence from GWAS studies reported the association of other SNPs within the CACNA1C and ANK3 genes but less consistently. For the CACNA1C, Sklar et al. (2011) reported a significant association of the CACNA1C rs4765913 with BD. For the ANK3, two SNPs have been found to be associated with BD, the ANK3 rs9804190 (Sklar et al., 2011; Baum et al., 2008) and the ANK3 rs1938526 (Dedman et al., 2012; Lee et al., 2011a; Takata et al., 2011).

4.4 The relevance of the CACNA1C and ANK3 risk genes in Bipolar Disorder

As mentioned in the previous paragraph, the most robustly identified SNPs associated with BD are located on chromosome 12p13 (CACNA1C rs1006737) and on chromosome 10q21.2 (ANK3 rs10994336 and rs9804190). Specifically, the ANK3 rs10994336 and the ANK3 rs9804190 are located at the 3' and the 5' end of the gene respectively and they lie very close to each other (about 340 kb apart in the gene). However, there is no evidence of linkage disequilibrium or other interaction between the corresponding SNPs (Schulze et al., 2009). These two regions are therefore considered as two independent genetic risk factors for BD. Finally, the risk alleles within the CACNA1C (rs1006737) and ANK3 (rs10994336 and rs9804190) risk genes lie in the intronic, non-coding regions of the genome and therefore they probably regulate the expression of the protein rather than change its structure. In line with this statement is the study by Quinn et al. (2010) which showed that these genetic variants are integral to gene expression regulation through cis- or trans-regulatory mechanism and therefore may alter the transcription of nearby or distant genes respectively. Moreover, their specific biological role, described in the paragraph below, may suggest a possible involvement of these genes in various aspect of neuronal development (Spitzer et al., 2006) that, consequently, may affect brain function and structure.

The biological functions of CACNA1C and ANK3 in brain neurons are linked to their effect on the efficiency of transmission of electrical impulses through the regulation of the voltage-gated ion channels. CACNA1C encodes for the alpha subunit of the L-type voltage dependent calcium (Ca^{+2}) channel Cav1.2. The ANK3 encodes for the Ankyrin G protein, found in the initial axon segment and the nodes of Ranvier, which are implicated in the generation and the amplification of action potentials (Lambert et al., 1997; Zhou et al., 1998). Therefore, the biological role of these genes on the neuronal level could have an effect on brain networks that are affected in BD patients, such as mood and cognition. Supporting this hypothesis is the evidence provided by several animal studies both for the CACNA1C and ANK3 genes. Specifically for the CACNA1C gene, Moosmang et al. (2005) reported an impairment in the hippocampus-dependent spatial memory in mice with an inactivation of the CACNA1C gene in the hippocampus and neocortex, by using two different spatial learning memory tasks (water maze and labyrinth-maze tasks), as well as dysfunctions in hippocampal synaptic plasticity. Disruptions in spatial memory have been found also by White et al. (2008) in Cav1.2 knock-out mice while performing a water maze task. In addition, Lee et al. (2012) and Dao et al. (2010) investigated the impact of the depletion of the CACNA1C in mice with relevance to human mood disorders. The authors showed that heterozygous CACNA1C knock-out mice presented decreased exploratory and antidepressant like behavioursas well as increased anxiety-related behaviour compared to wild type mice. Likewise, also the ANK3 gene has been study in transgenic mice that provided relevant insight into the effect of this gene on the neural network (Leussis et al., 2012). The authors suggested that in transgenic mice, in which brain-specific ANK3 isoforms were exclusively disrupted, showed changes in mood-related behaviours and elevated stress reactivity, functions that have been shown to be disrupted in BD patients (Leussis et al., 2012).

4.5 Population frequencies of the CACNA1C rs1006737, the ANK3 rs10994336 and the ANK3 rs9804190

The three SNPs within the CACNA1C and ANK3 genes are considered common genetic variants and therefore their frequency in the population is expected to be above 1%. In particular, several databases (dbSNP, Ensembl) for nucleotide variations are now available to provide important information about the minor allelic frequencies of several genetic

variants in the population, including the risk genetic variants employed in this thesis. For the CACNA1C rs1006737 risk gene (risk-allele A), the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/) reported that the minor allele is "A" and is present in 31% of European populations. For the ANK3 rs10994336 (risk-allele T), the minor allele is "T" and has a frequency of 12.3% whereas for the ANK3 rs9804190 (risk-allele C) the minor allele is "T" and has a frequency of 25.5%.

4.6 The effect of CACNA1C gene

Neuropsychological studies exploring the effect of the CACNA1C rs1006737 risk gene during processing of affective and non-affective cognitive tests

For the CACNA1C rs10096737 gene (risk-allele A), Soeiro-de-Souza et al. (2012) employed facial emotion recognition (FER) tests in a sample of 39 BD patients and 40 healthy individuals. The authors reported lower FER scores in BD patients homozygous for the CACNA1C rs1006737 risk-allele compared to carriers of only one risk-allele or the non-risk allelic variant. In 2013, the same group published a study investigating executive functions in a sample of 109 BD patients and 96 healthy individuals. The authors employed four executive functions tests (Letter-Number Sequence test, digit span, trail making test, and Wisconsin Card Sorting Test) and they reported that BD patients carriers of the risk-allele showed worse performance on all four executive function tests compared to BD patients carriers of the non-risk allele (Soeiro-de-Souza et al., 2013). In the same year, Arts et al. (2013) showed, in a sample of 51 BD patients, 34 first-degree relatives and 50 healthy individuals, poorer cognitive performance in several cognitive tasks in subjects homozygous for the risk variant of the CACNA1C rs1006737 gene, especially in the speed of processing task. Zhang et al. (2012) found significantly higher error rates on two working memory tasks, the N-back and the dot pattern expectancy tasks (DPX), in BD compared to healthy individuals independently from the genotype. During the 1-back and the DPX tasks, healthy individuals carriers of the risk-allele had higher error rates than subjects with the non-risk variant whereas BD patients showed an opposite direction of association. Finally, two studies in healthy individuals showed no effect of genotype on episodic memory and verbal intelligent (Erk et al., 2010) as well as on executive functions and verbal/learning memory tests (Roussos et al., 2011).

Evidence of the effect of CACNA1C rs1006737 risk gene in psychopathological measures and personality traits

Four studies have provided evidence of the effect of the CACNA1C rs1006737 gene on psychopathological aspects and personality traits (Erk et al., 2010; 2014; Roussos et al., 2011; Strohmaieret al., 2013). Erk et al. (2010) found that the CACNA1C rs1006737 risk-allele was associated with higher scores in depression, anxiety, obsessive-compulsive, interpersonal sensitivity and neuroticism. Similarly, the study by Erk et al. (2014) showed that RELs of BD patients had increased scores of anxiety measures. Roussos at al.(2011) reported that the risk-allele was significantly associated with low extraversion, higher harm avoidance, anxiety and paranoid ideation. Strohmaier et al. (2013) examined sex-specific effect of the CACNA1C rs1006737 gene in the population with a follow-up period of 10 years. In men, the risk-allele was associated with higher emotional lability and lower resilience, and more depressive symptoms at follow-up. In women, the risk-allele was associated with lower emotional liability and stronger resilience and fewer depressive symptoms at follow-up.

Neuroimaging studies exploring the effect of theCACNA1C rs1006737 risk gene

Functional Magnetic Resonance Imaging studies

Seven studies to date have looked into how the CACNA1C rs1006737 gene impacts brain function in healthy participants. During memory paradigms,the risk CACNA1C rs1006737 was associated with increased (Bigos et al., 2010) and decreased (Erk et al., 2014; Paulus et al., 2014) prefrontal activity and reduced hippocampal function (Erk et al., 2010; Krug et al., 2013). On the contrary, increased hippocampal activity was found during emotional processing in CACNA1C rs1006737 risk carriers (Bigos et al., 2010). Furthermore, increased activity in the right amygdala during a monetary reward paradigm was associated with the risk CACNA1C rs1006737 variant in healthy individuals (Wessa et al., 2010), as was increased activation in the left inferior frontal gyrus and precuneus during a semantic verbal fluency task (Krug et al., 2010). CACNA1C rs1006737 risk-allele carriers showed reduced neural activity in the right inferior parietal lobule during orienting and in the medial frontal gyrus during executive control of attention (Thimm et al., 2011).

Studies on the effect of the CACNA1C rs1006737 gene that include also BD patients and their RELs, have found increased right amygdala activity during a negative emotional faces task in CACNA1C rs1006737 risk carriers (Jogia et al., 2011; Tesli et al., 2013). In another study during an episodic memory task carrying the CACNA1C rs1006737 risk variant resulted in a stronger decrease of hippocampal and ACC activation in RELs, indicating an additive effect of CACNA1C rs1006737 variation on familial risk (Erk et al., 2014). Using Dynamic Causal Modelling (DCM) connectivity analysis, two studies found that, during emotional processing tasks, the presence of the CACNA1C rs1006737 risk-allele was associated with decreased effective connectivity between prefrontal and subcortical regions (Radua et al., 2013) as well as between visual and prefrontal cortices (Dima et al., 2013). In both studies the findings were significantly more marked in BD patients. Details of the studies have been reported in Table 4-2 and Table 4-3.

Table 4-2 FMRI studies exploring the effect of CACNA1C rs1006737 only in healthy individuals

Study	Participants (Male/Female)	Genotype	Age Mean, years (standard deviation)	Design	Contrast	Genetic Variant of CACNA1C (risk-allele A)	Exclusion Criteria
Bigos et al. (2010)	116 HI (56/60)	GG: 57 AG:43 AA: 16	29.37 (8.94) 29.47 (9.57) 27.88 (8.02)	Explicit memory task	Encoding and retrieval of aversive scenes > neutral scenes	rs1006737	No Axis I disorder
	131 HI (60/71)	GG: 64 AG:53 AA: 14	29.20 (8.78) 28.98 (8.84) 27.07 (7.98)	Emotional faces task	Emotional faces > neutral Faces		
	316 HI (146/170)	GG: 146 AG:141 AA: 29	30.92 (9.26) 31.48 (9.55) 30.07 (9.79)	Working memory task, (N-Back) Block	2-back > 0-back		
Erk et al. (2010)	110 HI (48/62)	GG: 60 AG:41 AA: 9	33.4 (10.7) 32.0 (9.5) 32.2 (10.1)	Episodic memory task, Block	Face profession pairs > baseline	rs1006737	No Axis I disorder
Erk et al. (2014)[1]	110 HI (See Erk et al. 2010) 59 BD RELs (23/36)	BD RELs: GG: 30 AG: 26 AA: 3	BD RELs: 31.8 (11.8)	Episodic memory task, Block	memory > control condition	rs1006737	No Axis I disorder
Erk et al. (2014)	Replication Sample: 179 HI (87/92) Combined Sample: 110 HI (See Erk et al., 2010)	GG: 81 AG:78 AA: 20	35.8 (9.6) 35.6 (9.2) 33.5 (9.5)	Episodic memory task, Block	Face profession pairs > baseline	rs1006737	No Axis I disorder

Study	Participants (Male/Female)	Genotype	Age Mean, years (standard deviation)	Design	Contrast	Genetic Variant of CACNA1C (risk-allele A)	Exclusion Criteria
Krug et al. (2010)	62 HI (62/0)	GG:32 AG + AA: 30	23.4 (3.8) 23.5 (2.6)	Semantic verbal fluency task, Block	Semantic verbal fluency >reading aloud Reading aloud > semantic verbal fluency	rs1006737	No Axis I disorder
Krug et al. (2013)	94 HI (66/28) 111 HI (68/43)	GG: 43 AG+AA: 51 GG: 58 AG+AA: 53	GG: 23.4 (3.3) AG+AA: 23.0 (2.5) GG: 33.7 (10.9) AG+AA: 32.8 (10.8)	Memory task (encoding and retrieval), Block	Encoding/retrieval > baseline	rs1006737	No Axis I disorder
Paulus et al. (2014)	96 HI (66/28)	GG: 43 AG: 37 AA: 12	GG: 23.4 (3.3) AG: 23.0 (2.8) AA: 23 (1.6)	N-back task, Block	2-back > 0-back	rs1006737	No Axis I disorder
Thimm et al. (2011)	80 HI (54/26)	GG: 36 AG: 34 AA: 10	GG: 23.3 (3.0) AG: 23.3 (3.2) AA: 23.1 (2.0)	Attention Network test, Event Related	orienting and executive control > baseline	rs1006737	No Axis I disorder
Wessa et al. (2010)	64 HI	GG: 31 AG: 26 AA: 7	GG: 26.09(8.43) AG+AA: 29.80 (13.67)	Monetary reward, Block	Reward > baseline	rs1006737	No Axis I disorder

BD= Bipolar Disorder; HI= Healthy Individuals; RELs= Unaffected first-degree relatives of BD patients.

[1]The study included also a sample of Relatives of Major Depressive Disorder and Schizophrenic patients.

Table 4-3 FMRI Studies exploring the effect of CACNA1C rs1006737 in BD

Study	Participants (Male/Female)	Genotype	Age Mean, years (standard deviation)	Design	Contrast	Genetic Variant of CACNA1C (risk-allele A)	Psychopathological measures (standard deviation)	Exclusion Criteria
Jogia et al. (2011)	41 euthymic BD (20/21) 25 RELs (13/12) 50 HI (27/23)	GG: 54 AG: 47 AA:15	BD: 44.29 (11.87) RELs: 35.0 (13.67) HI: 34.96 (13.20)	Facial Affect recognition task, Event-Related	Fear >neutral	rs1006737	HDRS: 4.77 (5.3) YMRS:1.44 (2.96) BPRS: 27.49 (3.99)	No other Axis I disorder
Dima et al. (2013)	41 euthymic BD (20/21) 46 HI (25/21)	GG: 45 AA+AG:42	BD: 44.3 (11.9) HI: 40.3 (13.2)	Facial affect recognition task, Event-Related	Fear, anger or sadness > neutral	rs1006737	HDRS: 4.8 (5.3) YMRS:1.4 (3.0) BPRS: 27.5 (4.0)	No other Axis I disorder
Tesli et al. (2013)[1]	66 euthymic BD 123 HI	N/A	N/A	Faces matching task (fear or anger), Block	Faces > shapes	rs1006737	YMRS:1.9 (3.5)	No other Axis I disorder

BD= Bipolar Disorder; BDI= Beck Depression Inventory; BPRS= Brief Psychiatric Rating Scale; HDRS= Hamilton Depressive rating scale; HI= Healthy Individuals; YMRS= Young Mania Rating Scale. [1] The study included also a sample of schizophrenic patients.

Structural studies

Six studies have been published exploring the role of CACNA1C rs1006737 risk-allele on the integrity of gray matter volumes. Four of them used only healthy individuals (Wang et al.,2011; Kempton et al.,2009; Franke et al., 2010; Erk et al., 2010)while the other two included also BD patients (Soeiro-de-Souza et al., 2012; Perrier et al., 2011). Two studies have found increased gray matter volume (Kempton et al., 2009) and increased total cortical volume (Wang et al., 2011) associated with the risk CACNA1C rs1006737 variant. Perrier et al. (2011) using a regions of interest approach found increased gray matter density in the right amygdala and hypothalamus in participants with the risk CACNA1C SNP, while another study found that the CACNA1C rs1006737 gene influences the brainstem rather than gray matter volume (Franke et al., 2010). Two studies found no influence of CACNA1C rs1006737 risk gene on hippocampal volumes in BD (Soeiro-de-Souza et al., 2012) and healthy individuals (Erk et al., 2010).

4.7 The effect of the ANK3 gene

Neuropsychological studies exploring the effect of the two allelic variations within the ANK3risk gene (rs10994336 and rs9804190) during processing of affective and non-affective cognitive tests.

For the ANK3 rs10994336 gene (risk-allele T), Ruberto et al. (2011) employed several neurocognitive tests (Degraded Symbol Continuous Performance Task, Stroop Colour Word Test, Wisconsin Card sorting test, the Wechsler Memory Scale and the Iowa Gambling Task) in a sample of 47 BD patients, 74 RELs and 67 healthy individuals. The authors showed that, although carriers of the risk-allele underperformed in all the tests, no significant differences between genotype and genotype by affection status were found, except for the Symbol Continuous Performance Task in which carriers of the risk variant showed decreased scores for false alarm and sensitivity. Hatzimanolis et al. (2012) reported that in the Continuous Performance Test-Identical Pairs, healthy individuals carriers of the ANK3 rs10994336 risk gene showed reduced sensitivity in target direction and significant more errors of commission compared to healthy individuals carriers of the common allele. No significant association has been found in either healthy individuals (Roussos et al., 2011; Hori et al., 2014) or BD patients (Hori et al., 2014) for this genetic variant in several cognitive domains.

Finally, for the ANK3 rs9804190 risk gene no neuropsychological evidence has been published yet.

Neuroimaging studies exploring the effect of the two allelic variations (rs10994336 and rs9804190) within the ANK3risk gene.

For the ANK3 gene only two studies investigated the effect of the ANK3 gene on brain activation (Dima et al., 2013; Roussos et al., 2012) and connectivity (Dima et al., 2013). During a facial affect labelling task, Dima et al. (2013) showed that the presence of the ANK3 rs10994336 risk-allele was associated with increased VLPFC activation in healthy individuals and reduced VLPFC activation in BD patients. Additionally, the presence of the ANK3 rs10994336 risk-allele was associated with decreased visual-prefrontal effective connectivity (Dima et al., 2013). The study by Roussos et al. (2012) explored the effect of the ANK3 rs9804190 risk gene in healthy individuals while processing the 3-back condition of the N-back task. The authors showed that carriers of the risk-allele showed increased activation in the left inferior frontal gyrus and in the left middle frontal gyrus (Table 4-4). Finally, a study by Linke et al. (2012) investigated the link between ANK3 rs10994336 risk gene and white matter integrity and showed that carriers of the risk-allele had reduced white matter integrity in the right anterior limb of internal capsule.

Table 4-4 FMRI Studies exploring the effect of the effect of the two allelic variations (rs10994336 and rs9804190) within the ANK3risk gene in BD and healthy individuals

Study	Participants (Male/Female)	Genotype	Age Mean, years (standard deviation)	Design	Contrast	Genetic Variant of ANK3	Psychopathological measures (standard deviation)	Exclusion Criteria
Dima et al. (2013)	41 euthymic BD (20/21) 46 HI (25/21)	CC: 57 CT+TT:30	BD: 44.3 (11.9) HI: 40.3 (13.2)	Facial affect recognition task, Event-Related	Fear, anger or sadness > neutral	rs10994336 (risk-allele T)	HDRS: 4.8 (5.3) YMRS:1.4 (3.0) BPRS: 27.5 (4.0)	No other Axis I disorder
Roussos et al. (2012)	52 HI (28/24)	CC: 31 CT+TT:21	HI (CC): 34.3 (13.4) HI (CT+TT): 36.5 (13.0)	Working memory task (N-back), Block	3-back vs 0-back	rs9804190 (risk-allele C)	N/A	No Axis I disorder

BD= Bipolar Disorder; BPRS= Brief Psychiatric Rating Scale; HDRS= Hamilton Depressive rating scale; HI= Healthy Individuals; YMRS= Young Mania Rating Scale.

4.8 Effect sizes of the risk variants within the CACNA1C and ANK3 risk genes on behavioural measures investigating emotion and cognition

The behavioural results suggest that CACNA1C rs1006737 and ANK3 rs10998336 risk alleles were associated with cognitive and emotional impairments. However, the overall effect-size of these differences oscillates between small and moderate suggesting that the overall strength of these differences is low. This is in line with the notion that common genetic variants have just small effects on clinical phenotypes and consequently we can expect that the CACNA1C rs1006737 and the ANK3 rs10994336 might have small effects also in behavioural measures linked to BD. This consideration might also explain the absence of neuropsychological evidence for the ANK3 rs9804190 and the negative results found by two studies which showed no effect of the two SNPs within the CACNA1C rs1006737 and ANK3 rs10998336 on episodic memory verbal intelligent (Erk et al., 2010) as well as on executive functions and verbal/learning memory tests (Roussos et al., 2011).

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5. The effect of *ANK3* bipolar-risk polymorphisms on the working memory circuitry differs between loci and according to risk-status for bipolar disorder

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Abstract

Polymorphisms at the rs10994336 and rs9804190 loci of the Ankyrin 3 (*ANK3*) gene have been strongly associated with increased risk for bipolar disorder (BD). However, their potential pathogenetic effect on BD-relevant neural circuits remains unknown. We examined the effect of BD-risk polymorphisms at rs10994336 and rs9804190 on the working memory (WM) circuit using functional magnetic resonance imaging (fMRI) data obtained from euthymic patients with BD (n=41), their psychiatrically healthy first-degree relatives (n=25) and unrelated individuals without personal or family history of psychiatric disorders (n=46) while performing the N-back task. In unrelated healthy individuals, the rs10994336-risk-allele was associated with reduced activation of the ventral visual cortical components of the WM circuit while the rs9804190-risk-allele was associated with inefficient engagement of the prefrontal cortical components of the WM. In patients and their healthy relatives, risk alleles at either loci were associated with hyperactivation in the ventral anterior cingulate cortex. Additionally, Rs9804190-risk-allele carriers with BD evidenced abnormal activation within the posterior cingulate cortex. This study provides new insights on the neurogenetic correlates of allelic variation at different genome-wide supported BD-risk associated *ANK3* locithat support their involvement in BDand highlight the modulatory influence of increased background genetic risk for BD.

Introduction

Allelic variation in the Ankyrin3 (*ANK3*) gene located on chromosome 10q21.2 has been most convincingly associated with increased risk for bipolar disorder (BD). The first report concerned a genome-wide association between BD and a single nucleotide polymorphism (SNP) at rs9804190 identified in two independent samples from the US and Germany (Baum et al., 2008). This association signal within a 70 kilobase region at the 3' end of the gene was later confirmed in a larger study by the Psychiatric GWAS Consortium Bipolar Disorder Working Group (Sklar et al., 2011). Three linked susceptibility loci at rs10994336 (Ferreira et al., 2008; Lett et al., 2011; Tesli et al., 2011), rs10994397 (Sklar et al., 2011) and rs1938526 (Takata et al., 2011; Lee et al., 2011; Dedman et al., 2012) have also been identified within a 250 kilobase region at the 5' end of the gene. The association signals within the 3' and 5' regions do not overlap and there is no evidence of linkage disequilibrium or other interaction between the corresponding SNPs (Schulze et al., 2009). These two regions are therefore considered as two independent genetic risk factors for BD.

The biological mechanisms linking allelic variation in the *ANK3* gene to increased risk for BD have yet to be clearly defined. The *ANK3* gene encodes for multiple protein isoforms of Ankyrin-G (AnkG) (Kordeli et al., 1995), a multi-functional protein with several distinct domains including spectrin- and trans-membrane binding domains. Brain-specific isoforms of AnkG are localized in the nodes of Ranvier and at axonal initial segments (AIS) (Kordeli et al., 1995). AnkG is involved in maintenance of neuronal polarity (Rasband, 2010) and in the clustering of ion gated channels required for action potential generation and propagation (Rasband, 2010; Zhou et al., 1998). Alterations in AnkG sequence or intracellular levels could disrupt these mechanisms and affect the function of neural circuits involved in mood and cognition. Congruent with this hypothesis, reduced *ANK3* expression of brain-specific transcripts in mouse models affects AIS throughout the brain (Leussis et al., 2013). These mice also exhibit a number of traits considered relevant to BD, specifically increased risk taking behaviour (decreased latency in the elevated plus maze and light-dark transition), greater reward salience (decreased latency to approach food in the novelty-suppressed feeding and increased sucrose preference), and increased reactivity to chronic stress (increased forced swim test immobility and elevated baseline and reactive corticosterone levels) (Leussis et al., 2013).

In human post-mortem samples, the BD-risk-alleles have been associated with reduced neuronal ANK3 expression in multiple brain regions (Roussos et al., 2012; Rueckert et al., 2013). However, in healthy individuals there are significant differences in the phenotypic traits associated with allelic variation at the 5' compared to the 3' *ANK3* region. Behaviourally, 5'risk-allele carriers (rs10994336) show increased anxiety-related temperamental traits (Roussos et al., 2011) while 3' risk-allele carriers (rs9804190) show abnormalities in psychosis-related traits (Roussos et al., 2012). White matter connectivity is reduced in 5'risk-allele carriers (rs10994336) but not in 3' risk-allele carriers (rs9804190) (Linke et al., 2012). In terms of cognitive function, 5'risk-allele carriers (rs10994336), but not 3' risk-allele carriers (rs9804190), underperform in tasks of sustained attention and set shifting (Linke et al., 2012; Ruberto et al., 2011; Hatzimanolis et al., 2012; Zhang et al., 2013). The one phenotypic trait shared by risk-alleles in both 3' and 5' *ANK3* regions is working memory disruption (Roussos et al., 2012; Ruberto et al., 2011) which is also a documented feature of BD.

Disruption in working memory (WM) circuitry in BD has been associated both with disease expression (Adler et al., 2004; Lagopoulos et al., 2007; Frangou et al., 2008; Townsend et al., 2010; Jogia et al., 2012; Pomarol-Clotet et al., 2012; Fernández-Corcuera et al., 2013) and familial risk (Drapier et al., 2008; Thermenos et al., 2010; Thermenos et al., 2011). Disease expression is associated with diminished function in dorsolateral frontoparietal regions involved in information encoding and maintenance (Adler et al., 2004; Lagopoulos et al., 2007; Frangou et al., 2008; Townsend et al., 2010; Jogia et al., 2012; Pomarol-Clotet et al., 2012; Fernández-Corcuera et al., 2013) and with failure to deactivate the default mode network (DMN) as evidenced by aberrant activation within medial prefrontal cortex and the anterior cingulate cortex (ACC) (Jogia et al., 2012; Pomarol-Clotet et al., 2012; Fernández-Corcuera et al., 2013). The failure to suppress ACC activation during the N-back has also been reported in unaffected first-degree relatives of patients and is likely to represent a genetically mediated vulnerability trait for BD (Drapier et al., 2008; Thermenos et al., 2010).

The current study examined the effect of SNP rs10994336 and rs9804190 on the neural circuitry subserving WM in a sample of 112 individuals comprising euthymic patients with BD (n=41), their psychiatrically healthy first-degree relatives (n=25) and unrelated healthy individuals (n=46). The study aimed to identify the neural mechanisms mediating the

increased risk for BD conferred by the two independent loci. We tested whether the pathogenetic effect of the risk-alleles at rs10994336 and rs9804190 independently contribute to failure to suppress DMC activation during the n-back task in patients and their healthy relatives, and whether a similar effect would be observed in unrelated individuals without a personal or family history of psychiatric disorders.

Subjects and methods

Participants

All participants were selected from the VIBES study cohort which comprises 75 families identified through a proband with BD type I and screened to exclude pedigrees with schizophrenia or schizophrenia spectrum disorders. Details of the VIBES rationale and design have been reported previously (Frangou, 2009). The sample considered in the present study comprised 41 euthymic patients with BD, 25 of their psychiatrically healthy first-degree relatives, and 46 healthy unrelated individuals, all of white British ancestry (Table 5-1). The study received institutional ethical approval. All individuals provided written informed consent prior to participation.

All participants were assessed by trained psychiatrists with patient or non-patient versions of the Structured Clinical Interview for Diagnosis (SCID) (First et al., 2002a,b), the Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960), the Young Mania Rating Scale (YMRS) (Young et al., 1978), the expanded Brief Psychiatric Rating Scale (BPRS) (Lukoff et al., 1986) and the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (Wechsler, 1981). Patients fulfilled criteria for BD type I based on the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, revised (American Psychiatric Association, 1994). We included only psychiatrically healthy relatives of BD probands based on the absence of a personal lifetime history of any psychiatric disorder. Unrelated healthy individuals without a personal or family history of psychiatric disorders were selected to match patients and relatives on age, sex, and IQ.

Exclusion criteria for all participants were current and hereditary neurological disorders, DSM-IV lifetime drug or alcohol dependence or drug or alcohol abuse in the preceding six months and contraindications to MR imaging. Prior to cognitive and MRI evaluation,

patients were required to have been in remission, defined as scoring below 7 in HDRS and YMRS, for a minimum of one month based on prospective weekly assessments, and to have remained on the same medication type and dose for at least six months. There was a significant effect of group on all symptom rating scales ($F_{(2,112)}$>9.82, p<0.001) with patients having higher scores than healthy relatives and unrelated healthy individuals; there was no difference between the latter two groups (Table 5-1). The HDRS, YMRS and BPRS were highly correlated with each other (all r=0.73, p<0.0001). As only the BPRS is suited for non-patient populations (healthy relatives and unrelated healthy individuals) this scale was chosen to control for psychopathology in subsequent analyses.

Thirty BD patients were on psychotropic medication; 12 on antipsychotics (7 on atypical, 2 on typical and 3 on both), 21 on mood stabilisers (lithium =15, sodium valproate=6), and 13 on selective serotonin reuptake inhibitors. None received anticholinergics or benzodiazepines. Medicated and unmedicated BD patients did not differ in age of onset, illness duration, IQ, HDRS, YMRS and BPRS total scores (all p> 0.31).

DNA extraction and genotyping

DNA was obtained from buccal swabs using conventional procedures. The *ANK3* rs10994336 (risk-allele T) as well as the *ANK3* rs9804190 (risk-allele C) genotype were determined by the TaqMan allelic discrimination assay (Applied Biosystems, Assay ID C_31344821_10). Endpoint analysis was performed using the Applied Biosystems 7900HT Fast Real-Time PCR System. Genotypes were called with the SDS 2.3 software and the output was checked visually to ensure genotypes fell into distinct clusters. Call rate was 100% as buccal swabs were repeated for 7 individuals for whom initial genotyping was undetermined. Accuracy was assessed by duplicating 15% of the sample. Reproducibility was 100%.

Within each group (patients, healthy relatives, unrelated healthy individuals) homozygote and heterozygote risk-allele carriers for each SNP were considered as detailed in Supplemental Tables 5-S1 and 5-S2. There was no effect of genotype or group-by-genotype interaction on age or sex (Tables 5-S1 and 5-S2).

Neuroimaging

Experimental Paradigm: The n-back task was employed in a block design incorporating alternating experimental and sensorimotor control conditions. A series of letters in yellow font were displayed on a blue screen for two seconds each. Participants were instructed to indicate by a button press whether the letter currently displayed matched the letter from the preceding n trials. In the sensorimotor control (0-back) condition, the letter "X" was the designated target. In the experimental conditions (1, 2, 3-back) the target letter was defined as any letter that was identical to the one presented in the preceding one, two, or three trials. There were 18 epochs in all, each lasting 30 seconds, comprising 14 letters with a ratio of target to non-target letters ranging from 2:12 to 4:10 per epoch. The entire experiment lasted 9 minutes and included a total of 49 target and 203 non-target stimuli. To avoid any systematic order effects the conditions were pseudo-randomised. Performance was evaluated in terms of reaction time to target letters and accuracy (% correct responses). Group differences in accuracy were examined using analysis of variance followed by pairwise comparisons with Bonferroni correction.

Acquisition Parameters: Gradient echo planar magnetic resonance (MR) images were acquired using a 1.5-Tesla GE Neuro-optimised Signa MR system (General Electric, Milwaukee, WI, USA) fitted with 40 mT/m highspeed gradients, at the Maudsley Hospital, London. Foam padding and a forehead strap were used to limit head motion. A quadrature birdcage head coil was used for radio frequency (RF) transmission and reception. A total of 180 T2*-weighted MR brain volumes depicting blood-oxygenation level-dependent (BOLD) contrast were acquired at each of 36 near-axial planes parallel to the inter-commissural (AC-PC) plane; repetition time (TR) = 3000ms, echo time (TE) = 40ms, slice thickness = 3mm, voxel dimensions = 3.75 x 3.75 x 3.30mm, interslice gap = 0.3mm, matrix size = 64 * 64, flip angle=90°. Prior to each acquisition sequence, four dummy data acquisition scans were performed to allow the scanner to reach a steady state in T1 contrast. During the same session, a high-resolution T1-weighted structural image was acquired in the axial plane for subsequent co-registration (inversion recovery prepared, spoiled gradient-echo sequence; TR = 18ms, TE = 5.1 ms, TI = 450 ms, slice thickness = 1.5 mm, voxel dimensions = 0.9375 × 0.9375 x 1.5 mm, matrix size 256 * 192, field of view = 240 x 180 mm, flip angle = 20°, number of excitations = 1.

Neuroimaging Data Analysis: All analyses were implemented using Statistical Parametric Mapping (SPM8) (www.fil.ion.ucl.ac.uk/spm/software/spm8/). The BOLD images were realigned to the fifth volume and corrected for interscan movements by means of a rigid body transformation with three rotation and three translation parameters. Subsequently, the 180 fMRI images were spatially normalized to the standard template of the Montreal Neurological Institute (MNI) and re-sampled to a voxel size of 2x2x2mm. Finally, the images were smoothed using an 8 mm full-width-half-maximum Gaussian kernel.

The smoothed single-subject images were analyzed via multiple regression using a standard linear convolution model, with vectors of onset representing the 1, 2, 3-back and the 0-back condition as the sensorimotor control. Serial correlations were removed using an AR(1) model. A high pass filter (128s) was applied to remove low-frequency noise.

As the effect of any single SNP on neural networks is expected to be subtle, all subsequent analyses were restricted to the 3-back condition because (a) individual differences in cognitive and neural efficiency are more apparent at high WM load (Gevins and Smith, 2000), and (b) the effect of diagnosis in patients with BD and their relatives is also most consistently seen at high WM load (Jogia et al., 2012; Palaniyappan and Liddle, 2014). Images representing the 3-back vs. 0-back contrast from each subject were entered in second level random-effects.

First, we investigated the main effect of each risk-SNP (rs10994336 and rs9804190) and their interaction on the WM circuitry in healthy unrelated individuals. This analysis allowed us to relate our findings to the literature that has examined the effect of *ANK3* only in unrelated healthy individuals. Second, full factorial ANCOVA was used to the effect of each SNP and their interactions in patients, healthy relatives and unrelated healthy individuals with BPRS and accuracy as covariates. Suprathreshold clusters were identified using Family Wise Error (FWE) correction of $P<0.05$. Stereotactic coordinates of the peak maxima of the suprathreshold clusters were converted (www.mrc-cbu.cam.ac.uk/Imaging/mnispace.html) from the Montreal Neurological Institute spatial array (www.mni.mcgill.ca) to that of Talairach and Tournoux (Talairach and Tournoux, 1988). Mean signal change from suprathreshold clusters was extracted using the MarsBaR toolbox (http://marsbar.sourceforge.net/) and was entered in correlation analyses to examine the

effect of age of onset, duration of illness, number of episodes, and medication dose at the time of scanning (lithium and antipsychotic). Threshold for statistical significance was set at $p< 0.005$ following Bonferroni correction.

Results

Effect of ANK3 allelic variation on clinical features

Rs10994336 or rs9804190 risk associated patients had significantly higher HDRS, YMRS and BPRS scores compared to all other groups ($F_{(2, 112)}>6.5$, $p<0.02$) (Supplemental Tables 5-S1 and 5-S2). There was no effect of genotype on patients' age of onset, duration of illness and number of mood episodes ($t_{39}<1.2$, $p>0.2$).

Effect of ANK3 allelic variation on cognitive task performance

There was no effect of group, genotype or group by genotype interaction for either SNP on general intellectual ability or response time ($p>0.1$)(Table 5-1, Supplemental Tables 5-S1 and 5-S2). In contrast, there was a significant effect of group on accuracy for the 3-back condition only, where relatives were significantly better than both other groups ($F_{(2,112)}>24.31$, $p<0.003$) (Table 5-1). Within the relatives group, non-risk associated relatives for either rs10994336 or rs9804190 had significantly higher accuracy ($p<0.01$) (Supplemental Tables 5-S1 and 5-S2).

Effect of ANK3 allelic variation on WM-related activation in unrelated healthy individuals

Healthy carriers of the rs10994336 risk-allele showed significantly decreased lateral temporal cortical activation within the middle (BA 21) and inferior (BA 20) temporal gyrus (Fig. 5-1). In contrast, healthy homozygotes of the rs9804190 risk-allele showed increased activation in the lateral prefrontal cortex within the inferior (BA 47/11) and middle (BA 46) frontal gyrus (Fig. 5-1). The coordinates of the peak height voxel of the corresponding suprathreshold clusters are presented in Table 5-2.

Effect of ANK3 allelic variation on WM-related activation in BD patients and their healthy relatives

An effect of group (patients, healthy relatives, healthy unrelated individuals) was observed in the middle (BA 9 and 10) frontal gyri, in the superior and middle temporal gyri (BA 21/22)

and in the ventral ACC (BA 24/32). When compared to unrelated healthy individuals, brain activation in patients was significantly (a) reduced in the left (BA 9) and right middle frontal gyri (BA 10) and, (b) increased in the superior and middle temporal gyri (BA 21/22) on the right and in the ACC bilaterally (BA 24/32). In comparison to patients, healthy relatives had greater activation in the middle frontal gyrus bilaterally. No differences were observed between unrelated healthy individuals and healthy relatives. The coordinates of the peak activations of the suprathreshold clusters are shown in Supplemental Table 5-S3.

In patients, there were no significant correlations between mean signal change in suprathreshold clusters and age of onset, duration of illness, mood episodes or medication dose ($p > 0.1$).

For the *ANK3rs10994336*, we found a significant group by genotype interaction in the right ventral ACC (x=4, y=19, z=-3, cluster size=35, z-value=3.67) and left ventral posterior cingulate cortex (PCC; x=-28, y=-64, z=16, cluster size=166, z-value=4.10). In the right ACC, the risk T-allele was associated with increased activation in BD patients and their healthy relatives compared to unrelated healthy individuals. In the left PCC, the risk T-allele was related with increased activation in BD patients compared to their healthy relatives and to unrelated healthy individuals (Fig. 5-2).

For the *ANK3 rs9804190*, a significant group by genotype interaction was found in the right ACC (x=4, y=17, z=-4; cluster size= 58; z-value=3.95) in which patients and healthy relatives who were risk C-allele homozygotes showed increased activation compared to unrelated healthy individuals (Fig. 5-2).

Discussion

There are two key findings from this study. First, in healthy individuals without personal or family history of psychiatric disorders, the rs10994336 and rs9804190 BD-risk alleles had different effects on the working memory (WM) network, although neither affected task performance. Second, both BD-risk alleles were associated with failure to deactivate the default mode network (DMN) in patients and in their healthy relatives; accuracy was reduced in risk-associated healthy relatives.

Unrelated healthy carriers of the *rs10994336* risk-allele showed reduced engagement of the ventral visual cortex within the middle and inferior temporal gyri (Table 5-2). This accords with previous reports from two independent samples which found that the largest effect size of the rs10994336 risk-allele was on reduced sensitivity in target detection and increased errors of commission during the degraded symbol continuous performance task (Ruberto et al., 2011; Hatzimanolis et al., 2012). Although the ventral visual cortex is an integral part of the WM circuitry, the core WM network involves the frontoparietal cortices (Owen et al., 2005; Leech et al., 2011; Rottschy et al., 2012). These regions are also core components of the superordinate cognitive control network that supports a broad range of executive function tasks (Niendam et al., 2012). Unrelated healthy rs9804190-risk allele homozygotes evidenced greater activation within the prefrontal components of the WM network although their task performance was comparable to that of the non-risk associated unrelated individuals (Table 5-2). This pattern is typically interpreted as evidence of cortical inefficiency, and is consistent with behavioural data from an independent sample that also found that healthy rs9804190-risk-allele homozygotes underperform in a wide array of executive function tasks (Roussos et al., 2012).

These findings suggest that in the absence of increased background genetic risk for BD or other psychiatric disorders the two *ANK3* BD-risk loci affect different regions of the WM circuitry. The reason for these regional differences is unclear. Available data suggest that 3' risk-alleles (rs9804190) are associated with reduced transcript levels of brain-specific AnkG isoforms. To date, the region most commonly implicated is the cerebellum where *ANK3* expression is generally highest (Rueckert et al., 2013). Information about other brain regions is incomplete because the available post-mortem studies have limited statistical power due to the small number of donors and provide incomplete brain coverage (Roussos et al., 2012; Rueckert et al., 2013). With regards to rs10994336, the effect of the risk-allele on *ANK3* expression in the brain is unknown. The rs10994336 polymorphism is located in an intronic region (Tesli et al., 2011) but could affect gene expression through cis- or trans-regulatory mechanisms (Quinn et al., 2010). Alternatively, rs10994336 may be in strong linkage disequilibrium with other, yet unidentified, genetic loci that drive the effects observed here.

Rs10994336 or rs9804190 risk-associated patients and relatives showed hyperactivity within the ventral ACC. The ventral ACC is integral to a network of brain regions involved in affect

processing and generation (Critchley et al., 2003) and a key component of the anterior DMN (Raichle et al., 2001; Buckner et al., 2008). Activation within the ventral ACC is increased during the processing of arousing stimuli or during mental stress (Critchley et al., 2003). The n-back task is quite challenging and may engender mild mental stress but it is not expected to result in ventral ACC hyperactivation. In fact, deactivation of the ventral ACC is normally observed during the n-back task within the context of anticorrelated activity between the DMN and the frontoparietal cognitive control network (Leech et al., 2011; Esposito et al., 2006).

Accordingly, healthy unrelated individuals in this study showed deactivation of the ventral ACC during the n-back task regardless of genotype. As expected, hyperactivation within the ventral ACC was observed in the patients regardless of genotype (Jogia et al., 2012; Pomarol-Clotet et al., 2012; Fernández-Corcuera et al., 2013) but it was more pronounced in rs10994336 or rs9804190 risk associated individuals. Amongst the healthy relatives, ventral ACC hyperactivity was only present in risk associated individuals for either risk-allele. Additionally, BD carriers of the rs10994336 risk-allele also showed hyperactivity within the PCC, centred on the ventral and extending to dorsal regions (Vogt et al., 2006). The PCC has dense anatomical connections with multiple cortical and subcortical regions (Hagmann et al., 2008) and is a core component of the posterior DMN (Raichle et al., 2001; Buckner et al., 2008). Healthy individuals performing the n-back task show deactivation in both dorsal and ventral PCC (Leech et al., 2011; Esposito et al., 2006) so the persistent PCC activation seen in patients suggests that the rs10994336 risk-allele compromises the ability to deactivate this brain region.

Taken together, these findings suggest that aberrant hyperactivation within the ventral ACC is a key mechanism mediating the risk-conferring effects of rs10994336 and rs9804190 in connection to the WM circuitry. It is noteworthy that this effect appeared to require the concomitant presence of additional risk factors for BD as it was not observed in unrelated individuals who had no personal or family history of such risk factors. This is consistent with the multifactorial pathogenetic model of BD that involves interaction between multiple genetic and non-genetic risk factors (Sullivan et al., 2012). The effect of any individual factor depends on the relative prevalence of other risk factors that are part of the same pathogenetic process. This observation is not unique to *ANK3.* Consistent with the findings

reported here, several neuroimaging studies have shown differential effects of various susceptibility polymorphisms (e.g. *DISC1, NRG1, COMT*) on brain structure and function in patients, high-risk groups and unrelated healthy individuals (Addington et al., 2007; Mechelli et al., 2008; Prata et al., 2008; Tsuchimine et al., 2013; Narr et al., 2009; Whalley et al., 2012).

In conclusion, our results point to a differential effect of BD-risk associated polymorphisms at ANK3 rs10994336 and rs9804190 modulated by risk-status for the disorder. This suggests that the BD-risk conferring mechanisms associated with these genetic variants are influenced by other genetic and possibly non-genetic factors that contribute to risk status. Inability to suppress key nodes of the DMN emerged as a common final pathway through which either risk-allele may contribute to the pathogenesis of BD. Mood stabilizing medications such as Lamotrigine interact with the ANK3 system through ion channels bound by AnkG to the axonal initial segment. Our results therefore lend further support to our previous study on patients with BD treated with Lamotrigine that showed “normalization” of the WM circuitry (Lang et al., 1993; Haldane et al., 2008) and suggest that ANK3-related molecular pathways may be a fruitful ground for the identification of new drug targets for BD.

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Table 5-1 Study Sample

	Unrelated Healthy Individuals N=46	Patients with BD N=41	Healthy Relatives N=25
Demographic Variables			
Age(years)	40.3 (13.2)	44.3 (11.9)	39.7 (13.7)
Sex (Male/Female)	25/21	20/21	13/12
Clinical Features			
HDRS total score [a]	0.1 (0.5)	4.8 (5.3)	0.14 (0.4)
YMRS total score [a]	0.2 (0.6)	1.4 (3.0)	0.0 (0.0)
BPRS total score [a]	24.3 (0.7)	27.5 (4.0)	24.1 (0.4)
Age of onset (years)	n/a	24.7 (8.0)	n/a
Duration of illness (years)	n/a	20.2 (10.5)	n/a
Depressive episodes (n)	n/a	5.7 (7.5)	n/a
Manic episodes (n)	n/a	5.6 (7.7)	n/a
Cognitive task performance			
IQ	112.6 (14.5)	117.9 (17.9)	115.8 (18.5)
1-back accuracy (% correct)	100	100	100
1-back response time (sec)	0.6 (0.3)	0.5 (0.2)	0.5 (0.2)
3-back accuracy (% correct)[b]	72.1 (17.2)	68.9 (19.7)	90.1 (15.4)
3-back response time (sec)	0.8 (0.4)	0.8 (0.3)	0.7 (0.2)

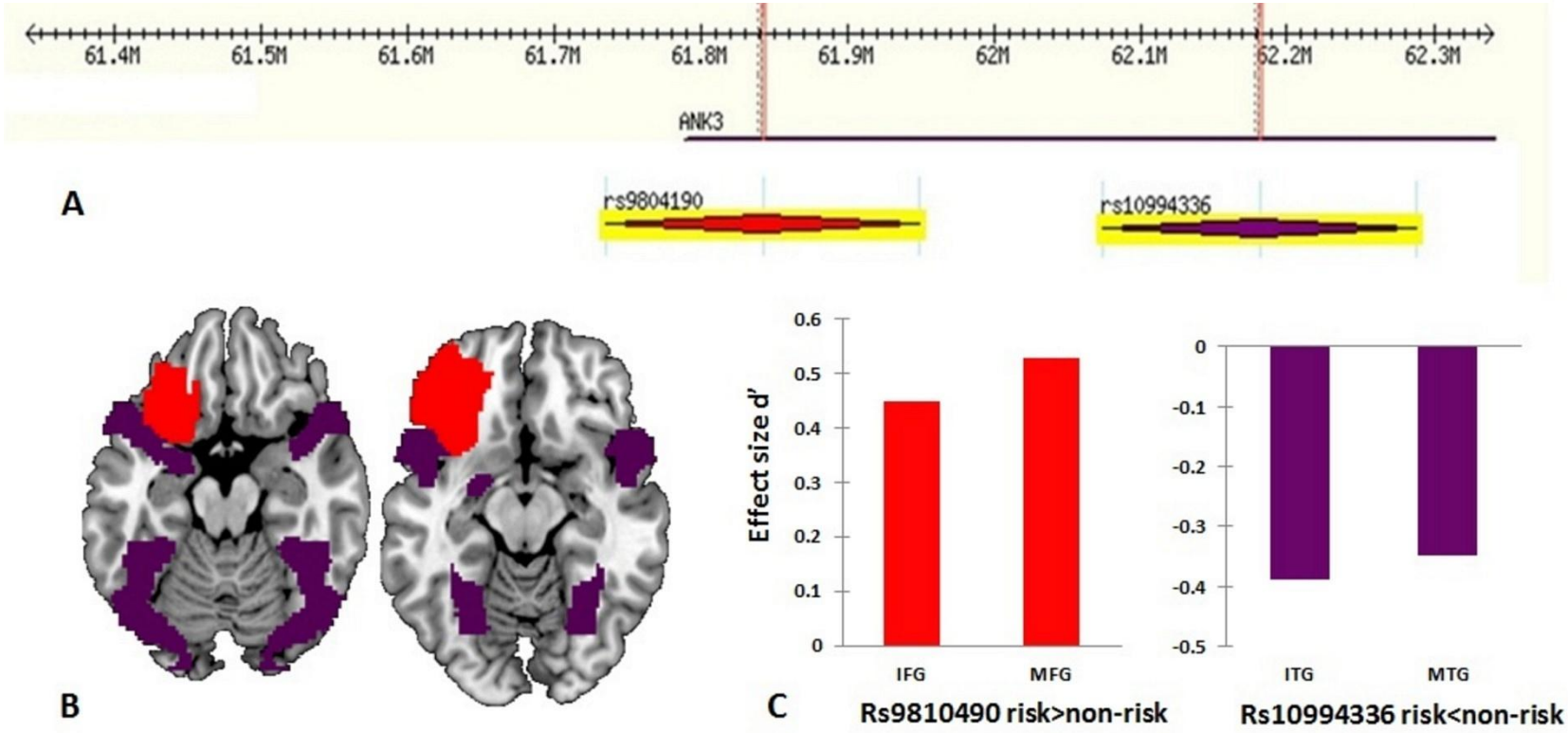
Except for sex, all data are presented as mean (standard deviation); **BD**= Bipolar Disorder; **BPRS**= Brief Psychiatric Rating Scale; **HDRS**=Hamilton Depression Rating Scale; **YMRS**=Young Mania Rating Scale. [a] Patients> healthy individuals and relatives ($P<0.001$); [b] Relatives> healthy individuals and patients ($P=0.003$).

Table 5-2 Brain regions showing significant effects of allelic variation at 10994336 and rs9810490 in the 3-back vs 0-back contrast in unrelated healthy individuals

Region	Gyrus	Laterality	Brodmann Area	Talairach and Tournoux Coordinates			Cluster size	z-value
				X	y	z		
ANK3 rs10994336: Risk-Allele Homozygotes < Non-Risk Allele Carriers								
Temporal	Middle Temporal	Left	21	-40	12	-28	120	3.41
		Right		48	-5	-15	90	3.76
				59	-14	-4	38	3.60
	Inferior Temporal	Left	20	-42	-12	-24	56	3.43
		Right		44	-11	-20	90	3.80
ANK3 rs9810490: Risk Allele Carriers > Non-Risk Allele Homozygotes								
Frontal	Middle Frontal	Left	46	-40	38	20	46	3.51
	Inferior Frontal	Left	47/10	-46	48	-4	51	3.48

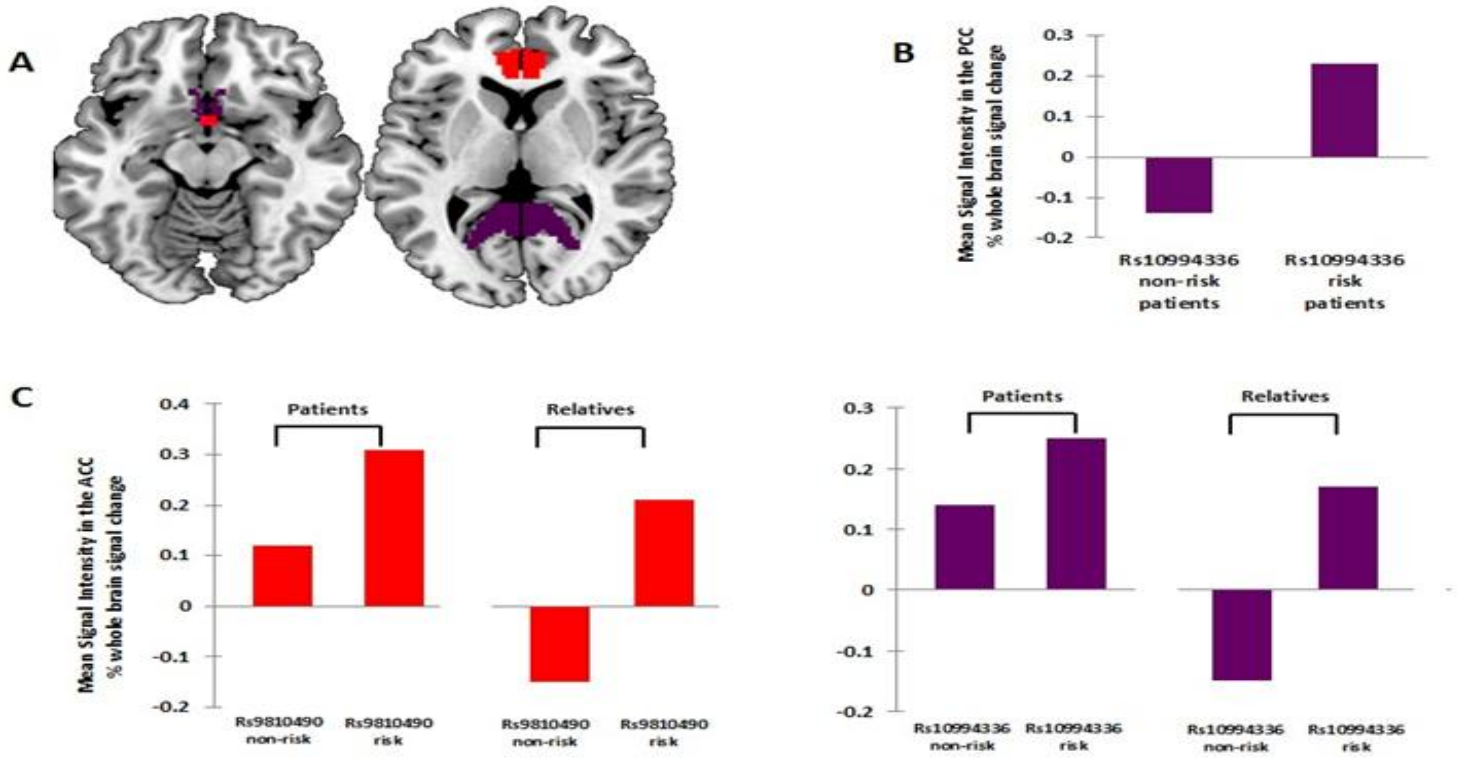
Suprathreshold clusters significant $p<0.05$ Family wise correction; x=sagittal; y=coronal; z=axial.

Figure 5-1 Effect of genotype on regional brain activation in the 3-back vs 0-back contrast in unrelated healthy individuals. All the regions showed are ROIs which I used only for graphical purposes to present brain regions significantly activated during the N-back task.



A. Chromosome 18: ANK3 rs984190 and rs10994336 loci. **B.** Rs984190 risk allele homozygotes show increased activation in the inferior (IFG) and middle (MFG) frontal gyrus (red) on the left; Rs10994336 risk allele carriers show decreased activation in the inferior (ITG) and middle (MTG) temporal gyri (purple) bilaterally. **C.** Effect size of mean signal change in the corresponding suprathreshold clusters; mean signal change in the temporal gyri was similar for left and right and was averaged.

Figure 5-2 Effect of genotype on regional brain activation in the 3-back vs 0-back contrast in patients with bipolar disorder and their psychiatrically healthy first-degree relatives.



A. Rs984190 (red) and Rs10994336 (purple) risk associated patients and relatives show increased activation in overlapping regions of the ventral anterior cingulate cortex (ACC); Additionally Rs10994336-risk associated patients showed increased activation in posterior cingulate cortex (PCC). **B.** Mean signal change in the PCC seen in patients only. **C.** Mean signal change in the ACC in patients and relatives.

Supplemental Material

Supplemental Table 5-S1 Effect of ANK3 rs10994336 genotype (risk-allele T)

	Effect of Genotype		Effect of Group by Genotype					
	Risk Associated TT+CT	No-risk Associated CC	Risk Associated TT+CT			No-risk Associated CC		
	N = 40	N = 72	Unrelated Healthy Individuals N = 14	BD patients N = 16	Healthy Relatives N=10	Unrelated Healthy Individuals N = 32	BD patients N = 25	Healthy Relatives n=15
Demographic Variables								
Age(years)	39.9 (13.1)	42.8 (12.28)	40.6 (12.2)	42.0 (10.7)	40.8 (8.3)	39.3 (12.3)	43.3 (12.3)	38.3 (13.7)
Sex (Male/Female)	21/19	34/38	7/7	9/7	4/6	18/14	11/14	7/8
Clinical Features								
HDRS total score [a]	2.1 (4.3)	0.5 (0.81)	0.4 (0.9)	5.3 (4.6)	0.3 (0.6)	0.1 (0.4)	1.5 (0.9)	0.1 (0.5)
YMRS total score [a]	0.7 (2.1)	0.1 (0.3)	0.2 (0.4)	1.6 (2.9)	0.0 (0.0)	0.2 (0.6)	0.7 (1.4)	0.0 (0.0)
BPRS total score [a]	25.6 (3.2)	24.9 (1.0)	24.8 (1.1)	27.3 (4.3)	24.7 (1.1)	24.2 (0.6)	25.9 (1.9)	24.1 (0.3)
Cognitive Performance								
IQ	117.2 (17.6)	116.9 (16.1)	110.7 (12.9)	112.3 (16.2)	112.0 (20.4)	116.7 (14.5)	121.7 (16.3)	118.3 (18.5)
3-back accuracy (%) [b, c]	78.7 (23.1)	66.9 (29.8)	70.5 (19.8)	73.3 (36.5)	83.8 (22.8)	75.1 (24.5)	67.2 (23.5)	90.78 (14.9)
3-back response time (sec)	0.84 (0.38)	0.77 (0.33)	0.99 (0.37)	0.68 (0.23)	0.54 (0.21)	0.86 (0.46)	0.94 (0.36)	0.75 (0.21)

Except for sex, all data are presented as mean (standard deviation); **BD**= Bipolar Disorder; **BPRS**= Brief Psychiatric Rating Scale; **HDRS**=Hamilton Depression Rating Scale; **YMRS**=Young Mania Rating Scale. [a] BD carriers of the risk-allele > unrelated healthy individuals, relatives, P<0.02; [b] relatives> unrelated healthy individuals, P=0.003; relatives> BD Patients, P = 0.003; [c] Group by Genotype Interaction, P = 0.01.

Supplemental Table 5-S2 Effect of ANK3 rs9810490 genotype (risk-allele C)

	Effect of Genotype		Effect of Group by Genotype					
	Risk Associated CC	No-risk Associated TT+CT	Risk Associated CC			No-risk Associated TT+TC		
	N = 63	N = 49	Unrelated Healthy Individuals N = 28	BD Patients N = 21	Healthy Relatives N = 14	Unrelated Healthy Controls N = 18	BD Patients N = 20	Healthy Relatives N = 11
Demographic Variables								
Age(years)	38.8 (13.6)	43.9 (12.1)	40.1 (13.26)	43.5 (12.51)	40.2 (14.3)	40.1 (11.9)	44.8 (9.5)	39.2 (13.8)
Sex (Male/Female)	29/34	29/20	14/14	8/13	7/7	11/8	12/9	6/5
Clinical Features								
HDRS total score [a]	1.7 (3.9)	2.1 (4.1)	0.1 (0.4)	5.5 (5.6)	0.1 (0.1)	1.2 (3.3)	4.5 (5.4)	0.07 (0.3)
YMRS total score [a]	0.7 (2.1)	0.5 (1.8)	0.2 (0.5)	2.0 (3.5)	0.0 (0.0)	0.06 (0.25)	1.2 (2.7)	0.0 (0.0)
BPRS total score [a]	25.8 (1.5)	25.1 (1.9)	24.3 (0.6)	29.3 (5.0)	24.2 (0.6)	24.7 (1.0)	26.3 (2.7)	24.1 (0.3)
Cognitive Performance								
IQ	111.8 (15.6)	120.4 (18.7)	117.6 (16.3)	114.1 (13.9)	108.5 (18.1)	119.4 (16.5)	118.3 (21.5)	122.9 (18.1)
3-back accuracy (%) [b, c]	72.02 (25.9)	83.01 (23.1)	67.1 (25.7)	73.5 (30.5)	85.6 (17.0)	85.8 (26.1)	62.5 (23.2)	92.5 (14.2)
3-back response time (sec)	0.76 (0.48)	0.58 (0.42)	0.85 (0.50)	0.61 (0.49)	0.70 (0.37)	0.81 (0.51)	0.41 (0.44)	0.60 (0.29)

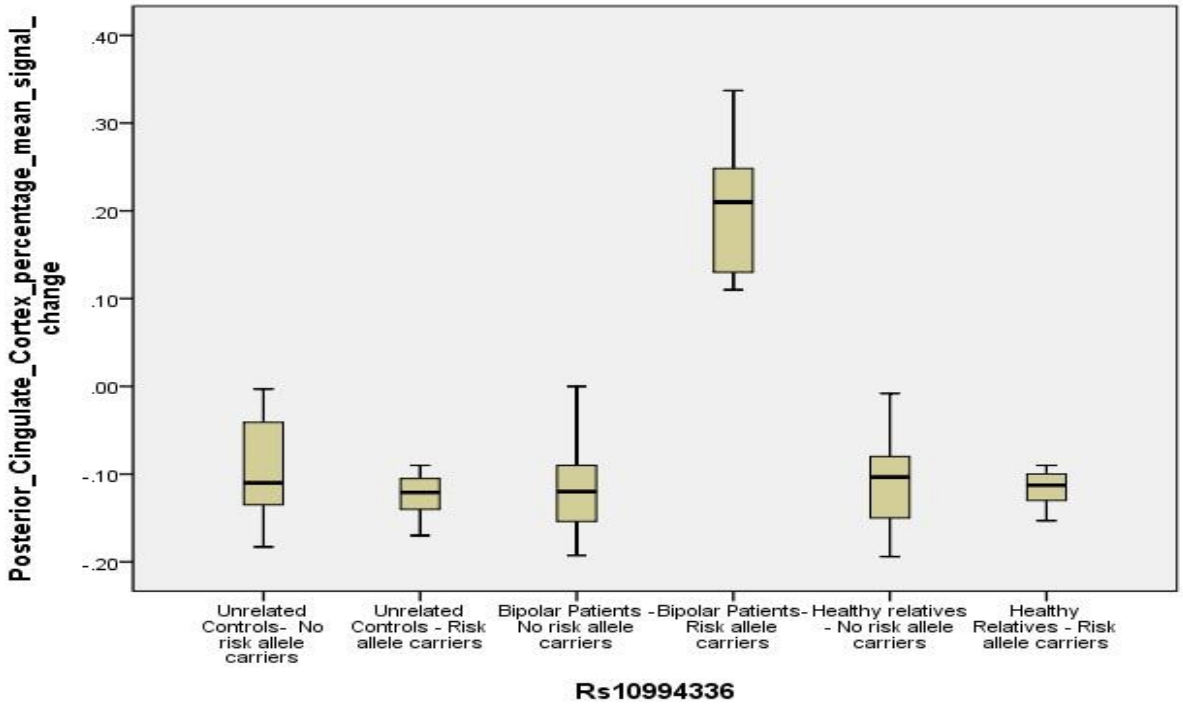
Except for sex, all data are presented as mean (standard deviation); **BD**= Bipolar Disorder; **BPRS**= Brief Psychiatric Rating Scale; **HDRS**=Hamilton Depression Rating Scale; **YMRS**=Young Mania Rating Scale. [a] BD carriers of the risk-allele > unrelated healthy individuals, relatives, P<0.02, relatives> unrelated healthy individuals, P = 0.003; relatives> BD Patients, P= 0.003; [c] Group by Genotype Interaction, P = 0.01.

Supplemental Table 5-S3 Brain regions showing significant effect of group in the 3-back vs 0-back contrast

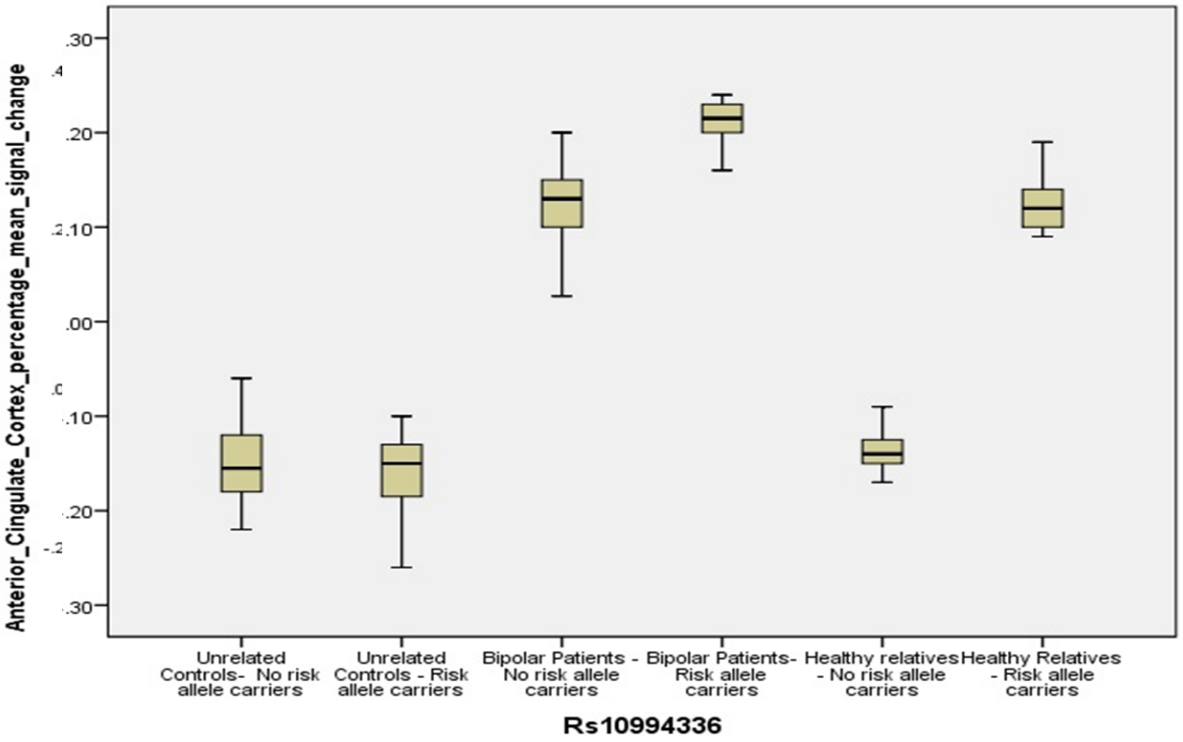
Gyrus	Laterality	Brodmann Area	Talairach and Tournoux Coordinates			z-value
			x	y	z	
Patients with BD > Healthy unrelated controls						
Anterior Cingulate	Left	24/32	-14	46	6	3.56
	Right		*10*	*26*	*-6*	*3.49*
Superior Temporal	Right	22	54	1	-4	3.93
Middle Temporal	Right	21	62	-8	-4	4.25
Patients with BD < Healthy unrelated controls						
Middle Frontal	Left	9	-34	14	40	3.70
	Right	10	38	56	-8	3.26
Patients with BD < Healthy relatives						
Middle Frontal	Left	9	-42	20	34	4.46
	Right	9	40	32	34	4.01

Suprathreshold clusters significant p<0.05 Family wise correction; x=sagittal; y=coronal; z=axial.

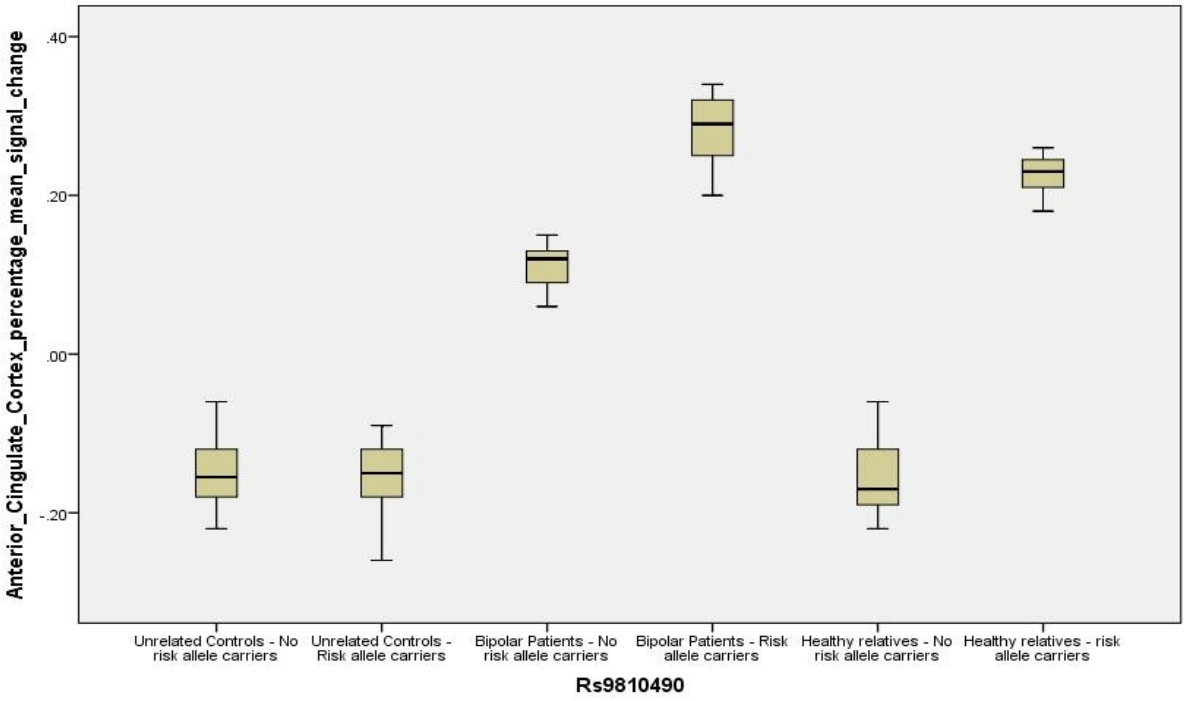
Supplemental Figure 5-S1 Box plot with the mean signal change in the posterior Cingulate Cortex in subjects carriying the ANK3 rs10994336.



Supplemental Figure 5-S2 Box plot with the mean signal change in the Anterior Cingulate Cortex in subjects carriying the ANK3 rs10994336.



Supplemental Figure 5-S3 Box plot with the mean signal change in the posterior Cingulate Cortex in subjects carriying the ANK3 rs9810490.



6. FMRI investigation on the effect of CACNA1C and ANK3 risk genes on brain regions during processing of facial affect labelling task in Bipolar disorder, their unaffected first-degree relatives and healthy individuals (in preparation for submission)

This chapter aims to explore the influence of genetic polymorphisms within the CACNA1C and ANK3 risk genes for bipolar disorder (BD) during processing of a facial affect labelling task in 46 healthy individuals, 41 BD patients and 25 of their first-degree healthy relatives. The groups employed in this study derived from the same cohort (VIBES) employed in the previous chapter.

Introduction

BD is a high heritable disease (85-89%, McGuffin et al., 2003) characterized by periods of depression and mania (American Psychiatric Association, 2013).In the last twenty years, functional magnetic resonance imaging (fMRI) studies have tried to identify the link between the clinical phenomenology of BD and the underlying neural systems that subserve emotional processing and regulation in BD across mood phases (manic, depressive and euthymic). A consensus model for BD suggests a dysfunction within distributed neural networks that underlie abnormal emotional processing in BD patients (Strakowski et al., 2012). Key regions within these networks involve the ventral prefrontal cortex (VPFC), the amygdala and other limbic areas (Chen et al., 2011). The pattern of activation in this network in BD patients seemed to be consistent across fMRI studies, with decreased activation in the VPFC (Altshuler et al., 2008; Jogia et al., 2008; Foland-Ross et al., 2012) and increased activation in the amygdala and other limbic associated regions(Foland et al., 2008; Jogia et al., 2008; Keener et al., 2012; Lawrence et al., 2004; Malhi et al., 2007).

Genome-wide association studies (GWAS) consistently identified several single-nucleotide polymorphisms (SNPs) associated with BD (Baum et al., 2008; Ferreira et al., 2008; Sklar et al., 2011). Among the most significant loci, GWAS studies identified genetic variants associated with BD within the ANK3, on chromosome 10q21.2 (rs10994336 and rs9804190; Ferreira et al., 2008; Sklar et al., 2011) and the CACNA1C, on chromosome 12p13 (rs1006737; Ferreira et al., 2008; Sklar et al., 2011). Evidence from animal studies reported

that these two genes have an effect on mood-related behaviours (Leussis et al., 2013; Shinnick-Gallagher et al., 2003). Specifically, the ANK3 gene encodes the ankyrin-G, a protein that is located at the nodes of Ranvier and the axonal initial segment and regulates the clustering of the voltage-gated sodium ion channels and neuronal excitability (Zhou et al., 1998; Pan et al., 2006). The CACNA1C gene encodes the alpha subunit of the L-type voltage-dependent calcium (Ca^{2+}) channel Cav1.2 and influences neuronal ability to generate and transmit electrical signals (Moosmang et al., 2005).

In healthy individuals, the CACNA1C rs1006737 risk-allele has been associated with increased amygdala activity (Bigos et al., 2010; Jogia et al., 2011; Wessa et al., 2010) and decreased connectivity between subcortical and prefrontal regions (Radua et al., 2012) as well as between visual and prefrontal cortices (Dima et al., 2013). For BD patients, two studies reported that the CACNA1C rs1006737 risk-allele was associated with significant decreased activation in the right VPFC compared to increased activation in healthy individuals (Dima et al., 2013; Jogia et al., 2011). Moreover, Jogia et al. (2011) reported that CACNA1C rs1006737 risk-allele was associated with significant increased activation of the VPFC in healthy relatives suggesting that this region may reflect the genetic pathway through which this BD-risk conferring SNP influence the emotional network in BD.

On the other hand, evidence from the effect of the genetic variations within the ANK3 gene are scarce. Only three fMRI studies explored the effect of the ANK3 rs10994336 or the ANK3 rs9804190 risk-alleles during processing of emotional or cognitive tasks (Dima et al., 2013; Delvecchio et al., 2015 (in press); Roussos et al., 2012). These studies suggested that both risk-alleles within the ANK3 gene modulate prefrontal regions, with reduced activation in the VPFC in BD patients and increased activation in healthy individuals during processing of emotional stimuli (Dima et al., 2013) as well as decreased activation in the inferior and middle frontal gyrus in healthy individuals during processing of a working memory task (Delvecchio et al., 2015 (in press); Roussos et al., 2012). Additionally, Delvecchio et al. (2015) also reported that BD patients and their healthy relatives carriers of either the ANK3 rs10994336 or the ANK3 rs9804190 risk-alleles showed increased activation in the ventral anterior cingulate cortex (ACC) compared to healthy individuals as well as increased activation in the posterior cingulate cortex in BD patients carriers of the ANK3 rs10994336 risk-allele compared to healthy individuals.

Based on these evidence, this study aimed to further explore the effect of CACNA1C rs1006737, ANK3 rs10994336 and ANK3 rs9804190 risk-alleles on brain activation during emotional processing in 41 euthymic BD patients, 25 of their healthy relatives and 46 healthy individuals. Because it has been shown that the facial affect is processed mainly by right sided regions, including the inferior occipital gyrus (IOG) and fusiform gyrus (FG), the amygdala (AMY) and the VPFC (Dima et al., 2011; Dima et al., 2013; Fairhall et al., 2007), we focused our investigation on these four volume of interests (VOIs) that also overlap with regions implicated in BD (Strakowski et al., 2012).

We tested the hypotheses that (a) the CACNA1C rs1006737, ANK3 rs10994336 and ANK3 rs9804190risk-alleles will independently contribute to modulate the neural responses within the four predefined VOIs of the facial affect processing network, (b) BD patients will exhibit decreased activation within the VPFC compared to healthy individuals and healthy relatives, and (c) the CACNA1C rs1006737, ANK3 rs10994336 and ANK3 rs9804190 risk-alleles will independentlyamplify the abnormal brain de-activation in the VPFC in BD patients.

Methods

Participants

All participants were selected from the VIBES study cohort which comprises 75 families identified through a proband with BD type I and screened to exclude pedigrees with schizophrenia or schizophrenia spectrum disorders. Details of the VIBES rationale and design have been reported previously (Frangou, 2009). The sample considered in the present study comprised 41 euthymic patients with BD, 25 of their psychiatrically healthy first-degree relatives, and 46 healthy unrelated individuals, all of white British ancestry (Table 6-1). The study received institutional ethical approval. All individuals provided written informed consent prior to participation.

All participants were assessed by trained psychiatrists with patient or non-patient versions of the Structured Clinical Interview for Interview (SCID) (First et al., 2002a, 2002b), the Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960), the Young Mania Rating Scale (YMRS) (Young et al., 1978), the expanded Brief Psychiatric Rating Scale (BPRS) (Lukoff et al., 1986) and the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (Wechsler, 1981). Patients

fulfilled criteria for BD type I based on the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, revised (American Psychiatric Association, 1994). Only psychiatrically healthy relatives were included based on the absence of a personal lifetime history of any psychiatric disorder. Unrelated healthy individuals without a personal or family history of psychiatric disorders were selected to match patients and relatives on age, sex, and IQ.

Exclusion criteria for all participants were current and hereditary neurological disorders, DSM-IV lifetime drug or alcohol dependence or drug or alcohol abuse in the preceding six months and contraindications to MR imaging. Prior to cognitive and MRI evaluation, patients were required to have been in remission, defined as scoring below 7 in HDRS and YMRS, for a minimum of one month based on prospective weekly assessments, and to have remained on the same medication type and dose for at least six months. There was a significant effect of group on in all symptom rating scales ($F_{(2,112)}$>9.82, P <0.001) with patients having higher scores than healthy relatives and unrelated healthy individuals; there was no difference between the latter two groups (Table 6-1). The HDRS, YMRS and BPRS were highly correlated with each other (all r=0.73, P <0.0001). As only the BPRS is suited for non-patient populations (healthy relatives and unrelated healthy individuals) this scale was chosen to control for psychopathology in subsequent analyses.

Thirty BD patients were on psychotropic medication; 12 on antipsychotics (7 on atypical, 2 on typical and 3 on both), 21 on mood stabilisers (lithium=15, sodium valproate=6), and 13 on selective serotonin reuptake inhibitors. None received anticholinergics or benzodiazepines. Medicated and unmedicated BD patients did not differ in age of onset, illness duration, IQ, HDRS, YMRS and BPRS total scores (all P > 0.31).

DNA extraction and genotyping

DNA was obtained from buccal swabs using conventional procedures. The *ANK3* rs10994336 (risk-allele T), the *ANK3* rs9804190 (risk-allele C) as well as the CACNA1C rs1006737 (risk-allele A) genotype were determined by the TaqMan allelic discrimination assay (Applied Biosystems, Assay ID C_31344821_10). Endpoint analysis was performed using the Applied Biosystems 7900HT Fast Real-Time PCR System. Genotypes were called with the SDS 2.3 software and the output was checked visually to ensure genotypes fell into distinct clusters. Call rate was 100% as buccal swabs were repeated for 7 individuals for whom initial

genotyping was undetermined. Accuracy was assessed by duplicating 15% of the sample. Reproducibility was 100%.

Within each group (patients, healthy relatives, unrelated healthy individuals) homozygote and heterozygote risk-allele carriers for each SNP were considered as detailed in Tables 6-2, Table 6-3 and Table 6-4. There was no effect of genotype or group-by-genotype interaction on age or sex.

Neuroimaging

Experimental Paradigm: The paradigm included 3 negative facial emotions (fear, anger, and sadness) in 3 separate experiments conducted in a single acquisition session in a randomized order. This paradigm consisted of 3 event-related tasks lasting 5 minutes each. In each task, 10 different facial identities (http://paulekman .com/) depicting 150% intensity of a negative (fear, anger, or sadness) or a neutral facial expression were presented in a pseudo random order interspersed with a fixation cross. The 150% level of intensity was chosen to minimize ambiguity about the nature of the stimuli. The stimuli (affective and neutral faces and the fixation cross) were each displayed for 2 seconds and repeated 20 times. The inter stimulus interval followed a Poisson distribution and varied between 3 and 9 (mean interval, 5) seconds. Participants were instructed to press the right or the left button with their dominant hand on an MRI compatible response box to indicate whether the face had an emotional or a neutral expression. Response time and accuracy data were collected.

Acquisition Parameters: Anatomical and functional imaging data were acquired during the same session using a 1.5-T MRI system (GE Sigma; General Electric). Gradient echo planar magnetic resonance (MR) images were acquired at each of the 16 noncontiguous planes parallel to the intercommissural (anterior commissure–posterior commissure) plane. I acquired T2*- weighted MR images reporting blood oxygenation level– dependent contrast (repetition time, 2000 milliseconds; echo time, 40 milliseconds; flip angle, 70°; section thickness, 7 mm; section skip, 0.7 mm; matrix size, 64 × 64; voxel dimensions, 3.75 × 3.75 × 7.7 mm). For each participant, 450 fMRIs were acquired. A high-resolution T1-lighted structural image was acquired in the axial plane for coregistration (inversion recovery-prepared, spoiled gradient-echo sequence; repetition time, 18 milliseconds; echo time, 5.1

milliseconds; inversion time, 450 milliseconds; flip angle, 20°; slice thickness, 1.5 mm; matrix size, 256 × 192; field of view, 240 × 180 mm; voxel dimensions, 0.9375 × 0.9375 × 1.5 mm; number of excitations, 1).

Demographic, Clinical and Task Performance Data Analysis: The groups were compared on demographic (age, gender and IQ) and clinical variables (HDRS, BPRS and YMRS total scores) using a univariate Analyses of Variance (ANOVA) with group or genotype as fixed factors.Similarly, task performance was examined using univariate ANOVA with accuracy or response time as dependent variables and group or genotype as independent factors. For all the statistical analyses, we controlled for possible confounding variables by including the BPRS total score as covariates.

Neuroimaging Data Analysis: All analyses were implemented using Statistical Parametric Mapping (SPM8) (www.fil.ion.ucl.ac.uk/spm/software/spm8/). The BOLD images were realigned to the fifth volume and corrected for interscan movements by means of a rigid body transformation with three rotation and three translation parameters. Subsequently, the 180 fMRI images were spatially normalized to the standard template of the Montreal Neurological Institute (MNI) and re-sampled to a voxel size of 2x2x2mm. Finally, the images were smoothed using an 8 mm full-width-half-maximum Gaussian kernel. For each participant, the fMRI data from the 3 event-related tasks (fear, anger, or sadness vs neutral) were concatenated and modelled with a general linear (convolution) model. Serial correlations were removed using an AR(1) model. A high pass filter (128s) was applied to remove low-frequency noise. Six movement parameters were also entered as nuisance covariates. The means of the 3 sessions were also modelled, as was the transition at the end of each session. For each participant, contrast images (affective > neutral facial expressions) were produced.

Group-level analyses were based on random-effects analyses of the single-subject contrast images using the summary statistic approach. To explore our hypotheses, four volumes of interest (VOIs) within the right IOG, FG, AMY and VPFC have been selected based on evidence of previous work that demonstrated the pivotal role of these regions during facial affect processing (Dima et al., 2011; Delvecchio et al., 2012; Dima et al., 2013). These VOIs were defined using a mask derived from the automated anatomical labeling atlas in Wake

Forest University PickAtlas (version 3.0.3;www.fmri.wfubmc.edu/software/PickAtlas). Measures of brain activation (weighted parameter estimates) (Kiebel et al., 2007) were extracted for each VOI from one-sample *t*-tests (contrast images affective>neutral facial expressions) for each group using a region-of-interest toolbox for SPM (MarsBaR; http://marsbar.sourceforge.net).

These measures were analyzed in SPSS (version 20; SPSS, Inc). First, a multivariate ANOVA was performed to explore the effect of group on the four predefined VOIs (dependent variable: extracted weighted parameter estimates for each VOI; independent factors: group). Second, a two-sample *t*-test was carried out to investigate the independent effect of each genotype on neural activation in the four predefined VOIs. Third, a multivariate ANOVA was performed to explore the group by genotype interaction for each genotype separately (dependent variable: extracted weighted parameter estimates for each VOI; independent factors: group and genotype). All results were corrected by using the Bonferroni correction. The new critical value (a=0.01) for an individual test was found by dividing the family-wise error rate (0.05) by the number of tests employed (4). Threshold for statistical significance was then set at p< 0.01 following Bonferroni correction.

Results

Demographic and clinical data

There was no effect of genotype or group by genotype interaction on demographic variables (*P*>0.2). However, BD patients, regardless of genotype, showed higher symptoms scores compared to all the other groups; rs10994336, rs9804190 or rs1006737 risk associated patients had significantly higher HDRS, YMRS and BPRS scores compared to all other groups (P<0.02) (Table 6-2, Table 6-3, Table 6-4).

Task performance

There was no effect of group, genotype or group by genotype interaction for either SNP within the ANK3 and CACNA1C genes on accuracy (*P*>0.6) (Table 6-2, Table 6-3, Table 6-4). BD patients had longer mean response time compared to their healthy relatives and healthy individuals (P<0.002) but there was no effect of genotype nor an interaction between group (BD patients, healthy relatives, healthy individuals) and genotype (all P>0.54).

Neuroimaging

No significant suprathreshold clusters were found in the whole-brain analyses for each genotype.

Multivariate ANOVA in the three groups regardless of genotype

Multivariate ANOVA with weighted parameters from the four VOIs as dependent variables and group as independent factor showed a significant effect of group in the VPFC (all $F_{(2,112)}$>9.5, all P<0.0001). Post hoc analyses showed that mean levels of activation were reduced in BD patients and healthy individuals compared to healthy relatives (P< 0.001). However, BD patients and healthy individuals did not significantly differ from each other (P> 0.2) (Fig. 6-1).

Two-sample *t*-test exploring the effect of each genotype regardless of group

Two-sample *t*-tests with weighted parameters from the four VOIs as dependent variables and genotype as grouping factor showed the ANK3 rs10994336 and CACNA1C rs1006737 risk-alleles were independently associated with greater activation in all four predefined VOIs, including the IOG, FG, AMY, and VPFC, while the ANK3 rs9804190 risk-allele carriers had an opposite pattern of activation (P< 0.05) (Fig. 6-2).

Multivariate analysis of variance exploring the group by genotype interaction in the three genotypes separately

For each genotype, we found a significant group by genotype interaction in the VPFC (all $F_{(2,112)}$> 4, all P<0.001). The results showed that the ANK3 rs10994336, the CACNA1C rs1006737 and the ANK3 rs9804190 risk-alleles were independently associated with decreased activation in the VPFC in BD patients. In contrast, in healthy individuals and healthy relatives the ANK3 rs10994336 or the CACNA1C rs1006737 risk-alleles were associated with increased activation in this brain region whereas the ANK3 rs9804190 risk-allele carriers showed the opposite pattern of activation(all P<0.0015) (Fig. 6-3).

Discussion

It has been reported that psychiatric disorders, including BD, involved complex and polygenic modes of inheritance in which each gene might have only a small effect. Therefore, the lack of significant results found in the whole-brain analyses might be due to

the subtle effect of any single SNP on the neural networks. For this reason, to increase the power to detect the effect of any single SNP on brain activation we to extracted the BOLD signal from four VOIs that have been shown to have a pivotal role in the facial affect processing (Dima et al., 2013) and to perform statistics across only these regions. This approach was used in order to reduce the severity of correction for multiple tests by correcting only for a small number of VOIs instead of correcting for the large number of voxels in the brain (Poldrack et al., 2007).

In this study the effect of three SNPs within the ANK3 (rs10994336, rs9840190) and CACNA1C (rs1006737) risk genes on neural correlates of facial affect processing was explored using a VOIs approach in BD patients, their healthy relatives and healthy individuals. The key findings of this study are fourfold (a) a significant main effect of group was found in the VPFC, (b) a main effect of genotype was found in all four predefined VOIs with the ANK3 rs10994336 and the CACNA1C 1006737 risk-alleles showing the same effect on these regions compared to the ANK3 rs9804190 risk-allele, (c) a significant group by genotype interaction was found in the VPFC for each genotype independently, with the ANK3 rs10994336 and the CACNA1C rs1006737 risk-alleles showing the same modulation effect on the VPFC in the three groups compared to the ANK3 rs9804190 risk-allele.

Independent effect of each genetic variation within the CACNA1C and ANK3 genes on facial affect processing network

Results of this study supported the initial prediction that the three genotypes, regardless of group, independently modulated the brain activation in key regions of the facial affect processing network (Fig. 6-2). Specifically, we found that the ANK3 rs10994336 and CACNA1C rs1006737 risk-alleles were independently associated with increased activation in the four predefined VOIs. These findings partially replicated the results of a previous study by Dima et al. (2013) that reported increased activation in the IOG, AMY, FG in both healthy individuals and BD patients carriers of the risk-alleles within the CACNA1C rs1006737 and ANK3 rs10994336 genes. Moreover, these results are also in keeping with the evidence from two previous fMRI studies which reported that the CACNA1C rs1006737 significantly increased the activation of the AMY (Wessa et al., 2010; Jogia et al., 2011) and the VPFC (Jogia et al., 2011) in both healthy individuals and healthy relatives.The interpretation of these results is still unclear especially because the function of non-coding intronic SNPs,

such as the ones explored in this study, within the genome is still under investigation. However, what we do know is that since many of the GWAS hits for common diseases, including BD, lie in the non-coding regions of the genome it is likely that variation in these areas have an effect on gene expression (Hindorff et al., 2009; Chorev et al., 2012), including the genetic variants within the ANK3 and CACNA1C genes (Quinn et al., 2010). Moreover, because the CACNA1C and ANK3 genes regulate the voltage-gated ion channels that are involved in various aspect of neuronal development (Spitzer et al., 2006), alterations in gene expression may therefore affect brain structure, which could also affect brain function.

Additionally, for the ANK3 rs9804190 risk-allele we observed the opposite pattern of activation, with risk-allele carriers showing a decreased activation in the four predefined VOIs. Also in this case, the reason for these differences, especially between the two SNPs within the same ANK3 gene, is still unclear as no fMRI studies exploring the effect of the ANK3 rs9894190 risk-allele on brain activation during emotional processing have been published yet.The rs10994336 and rs9894190 are over 340 kb apart and at the 5' and 3' end of the ANK3 gene respectively and with no evidence of linkage disequilibrium (LD) (Schulze et al., 2009). LD is defined as the non random association of alleles of two or more loci (Slatkin et al., 2008) and it is used to identify redundant tracts of the genome that carry the same genetic information (Meadows et al., 2008). More importantly, it has been reported that differences in LD can lead to differences in the effect of a SNP in different populations (Bastiaansen et al., 2014). Therefore, it may be plausible that the ANK3 rs10994336 and the ANK3 rs9840190 subserve different functions on molecular level and therefore may impact brain development or brain activity in different ways yet to discover.

The role of the VPFC in the pathophysiology of BD

Our results also fully support our initial prediction that BD patients would have a significant decreased activation in the VPFC compared to healthy individuals and healthy relatives (Fig. 6-1) and also that the three risk-alleles within the CACNA1C and ANK3 genes would independently amplified this abnormal brain de-activation (Fig. 6-3). The VPFC has been considered the core regulatory centre of emotions (Ochner et al., 2005; Phillips et al., 2008) that has direct anatomical connections with the amygdala (Ray and Zald, 2012). The involvement of this area during emotional processing has been reported by prior fMRI

studies in healthy individuals that suggested the pivotal role of this region in the voluntary suppression of affect that could represent increasing effectiveness in emotion regulation (Phan et al., 2005; Levesque et al., 2003). Therefore, decreased activation in the VPFC found in BD patients may be considered as a dysfunction of the VPFC in exerting cognitive or inhibitory control on emotional response's areas (Hariri et al., 2003). This finding confirmed the evidence from previous meta-analyses that reported reduced activation in this region (Houenou et al., 2011; Delvecchio et al., 2012), especially when BD patients processed negatively valenced emotions (Delvecchio et al., 2012).

Finally, our results also showed a significant group by genotype interaction in the VLPFC for the ANK3 rs9804190, the ANK3 rs10994336 and the CACNA1C rs1006737 (Fig 6-3). We found thathealthy individuals and healthy relatives carriers of the CACNA1C rs1006737 or the ANK3 rs10994336 BD-risk conferring SNPs had increased activation in the VPFC. These findings are in line with the evidence that genes have more penetrant power in detecting risk-associated alterations in brain function than clinical diagnoses (Hariri and Weinberg, 2003) and therefore can be identified also in healthy risk-allele carriers. Our results can be interpreted as an indicator of prefrontal physiological inefficiency related to genetic risk (Callicott et al., 2003a). In other words, the increased activation of the VPFC in unaffected individuals might be considered in the light of an exaggerated, compensatory mechanism of the top-down cognitive modulation exerted by the VPFC over brain regions known to be involved in emotional processing, such as the amygdala (Jogia et al., 2012). Therefore, overactivation in this area may underlie and reflect a compensatory effect and a capacity for resilience of unaffected individuals in response to BD-related disturbance in the VPFC.This interpretation might be supported by a functional connectivity study by Pompei et al. (2011b) that found a significant positive connectivity between VPFC and the dorsal PFC in healthy relatives during the Stroop task. The authors reported that the increased dorso-ventral prefrontal connectivity is related to an increased in task difficulty and therefore it may reflect a capacity for resilience in this group who engaged more prefrontal regions to compensate dysfunctions in other brain regions that are usually involved in a response inhibition task.

In contrast, we found that the ANK3 rs9804190 risk-allele was associated with decreased activation in the VPFC in healthy individuals and healthy relatives, thereforeshowingan

opposite pattern of activation compared to the ANK3 rs10994336 and CACNA1C rs1006737 risk-alleles. This finding is in line with a previous fMRI study (Delvecchio et al., 2015,in press) that explored the effect of the ANK3 rs10994336 and the ANK3 rs9804190 during a working memory task. The authors showed that in healthy individuals, the ANK3 rs9804190 risk-allele had not only a different impact on neural activation but also modulated completely different brain regions compared to the ANK3 rs10994336 risk-allele. Nonetheless, the reason for these differences is still unclear especially because our knowledge of ANK3 expression in the brain is still limited.

Conclusions

Taken together, these findings showed that the three genetic variants within the CACNA1C and ANK3 genes are associated with functional effects on human brain not only in BD patients but also in individuals who do not show an expression of BD, e.g. healthy individuals and healthy relatives. The effect of the ANK3 rs9804190, ANK3 rs10994336 and CACNA1C rs1006737 risk-alleles is more prominent in the VPFC, a core region of the emotional brain network, in all three groups. Moreover, the decreased activation of the VPFC in our sample of remitted BD patients, with and without the presence of genetic risk, is in line with prior hypotheses supporting that the abnormal activation in the VPFC is not only related to either the depressive or manic state but also to the euthymic phase of the illness and therefore a stable trait of BD (Townsend et al., 2012). Finally, we cannot exclude that our results may be due to the influence of other genetic or environmental factors interacting with each other (Geoffroy et al., 2013). In support of this statement is also the study by Cooper et al. (2013) that highlighted that the reason why healthy individuals harbour potentially disadvantageous variants but without suffering any obvious ill effects might be due to several reasons including the possible requirement of additional genetic and/or environmental factors to its manifestation.

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Table 6-1 Study Sample

	Unrelated Healthy Individuals N=46	Patients with BD N=41	Healthy Relatives N=25
Demographic Variables			
Age(years)	40.3 (13.2)	44.3 (11.9)	39.7 (13.7)
Sex (Male/Female)	25/21	20/21	13/12
Clinical Features			
HDRS total score [a]	0.1 (0.5)	4.8 (5.3)	0.14 (0.4)
YMRS total score [a]	0.2 (0.6)	1.4 (3.0)	0.0 (0.0)
BPRS total score [a]	24.3 (0.7)	27.5 (4.0)	24.1 (0.4)
Age of onset (years)	n/a	24.7 (8.0)	n/a
Duration of illness (years)	n/a	20.2 (10.5)	n/a
Depressive episodes (n)	n/a	5.7 (7.5)	n/a
Manic episodes (n)	n/a	5.6 (7.7)	n/a
Cognitive task performance			
IQ	112.6 (14.5)	117.9 (17.9)	115.8 (18.5)
Correctly identified faces, %	93.1 (4.8)	90.3 (4.1)	89.9 (8.4)
Response time, ms[b]	1109 (241)	1491 (209)	1107 (152)

Except for sex, all data are presented as mean (standard deviation); **BD**= Bipolar Disorder; **BPRS**= Brief Psychiatric Rating Scale; **HDRS**=Hamilton Depression Rating Scale; **YMRS**=Young Mania Rating Scale.[a] Patients> healthy individuals and relatives (P<0.001); [b] Relatives and healthy individuals> patients (P<0.003).

Table 6-2 Effect of ANK3 rs10994336 genotype (risk-allele T)

	Effect of Genotype		Effect of Group by Genotype					
	Risk Associated TT+CT	No-risk Associated CC	Risk Associated TT+CT			No-risk Associated CC		
	N = 40	N = 72	Unrelated Healthy Individuals N = 14	BD patients N = 16	Healthy Relatives N = 10	Unrelated Healthy Individuals N = 32	Patients with BD N = 25	Healthy Relatives N = 15
Demographic Variables								
Age(years)	39.9 (13.1)	42.8 (12.3)	40.6 (12.2)	42.0 (10.7)	40.8 (8.3)	39.3 (12.3)	43.3 (12.3)	38.3 (13.7)
Sex (Male/Female)	21/19	34/38	7/7	9/7	4/6	18/14	11/14	7/8
Clinical Features								
HDRS total score[a,b]	2.1 (4.3)	0.5 (0.8)	0.4 (0.9)	5.3 (4.6)	0.3 (0.6)	0.1 (0.4)	1.5 (0.9)	0.1 (0.5)
YMRS total score [a,b]	0.7 (2.1)	0.1 (0.3)	0.2 (0.4)	1.6 (2.9)	0.0 (0.0)	0.2 (0.6)	0.7 (1.4)	0.0 (0.0)
BPRS total score [a,b]	25.6 (3.2)	24.9 (1.0)	24.8 (1.1)	27.3 (4.3)	24.7 (1.1)	24.2 (0.6)	25.9 (1.9)	24.1 (0.3)
Cognitive Performance								
IQ	117.2	116.9	110.7 (12.9)	112.3	112.0 (20.4)	116.7 (14.5)	121.7 (16.3)	118.3 (18.5)
Correctly identified faces, %	90.2 (10.2)	89.6 (11.1)	95.0 (4.1)	91.2 (6.2)	88.01 (13.0)	92.8 (7.0)	90.0 (9.4)	90 (8.3)
Response time, ms[b]	1248 (237)	`1137 (206)	1193(328)	1244 (221)	1196 (41)	1096 (185)	1252 (249)	1092 (148)

Except for sex, all data are presented as mean (standard deviation); **BD=** Bipolar Disorder; **BPRS=** Brief Psychiatric Rating Scale; **HDRS=** Hamilton Depression Rating Scale; **YMRS=** Young Mania Rating Scale. [a] Scores for BD patients are significantly greater than those for healthy individuals and unaffected first-degree relatives ($P < .02$); [b] Scores for BD patients are significantly greater than those for all other groups ($P < .02$).

Table 6-3 Effect of ANK3 rs9810490 genotype (risk-allele C)

	Effect of Genotype		Effect of Group by Genotype					
	Risk Associated CC	No-risk Associated TT+CT	Risk Associated CC			No-risk Associated TT+TC		
	N = 63	N = 49	Unrelated Healthy Individuals N = 28	BD Patients N = 21	Healthy Relatives N= 14	Unrelated Healthy Individuals N = 18	BD Patients N = 20	Healthy Relatives N = 11
Demographic Variables								
Age(years)	38.8 (13.6)	43.9 (12.1)	40.1 (13.26)	43.5 (12.51)	40.2 (14.3)	40.1 (11.9)	44.8 (9.5)	39.2 (13.8)
Sex (Male/Female)	29/34	29/20	14/14	8/13	7/7	11/8	12/9	6/5
Clinical Features								
HDRS total score [a,b]	1.7 (3.9)	2.1 (4.1)	0.1 (0.4)	5.5 (5.6)	0.1 (0.1)	1.2 (3.3)	4.5 (5.4)	0.07 (0.3)
YMRS total score [a,b]	0.7 (2.1)	0.5 (1.8)	0.2 (0.5)	2.0 (3.5)	0.0 (0.0)	0.06 (0.25)	1.2 (2.7)	0.0 (0.0)
BPRS total score [a,b]	25.8 (1.5)	25.1 (1.9)	24.3 (0.6)	29.3 (5.0)	24.2 (0.6)	24.7 (1.0)	26.3 (2.7)	24.1 (0.3)
Cognitive Performance								
IQ	111.8 (15.6)	120.4 (18.7)	117.6 (16.3)	114.1 (13.9)	108.5 (18.1)	119.4 (16.5)	118.3 (21.5)	122.9 (18.1)
Correctly identified faces, %	90.4 (12.8)	88.4 (9.4)	92.38 (6.9)	87.2 (12.7)	91.3 (6.9)	90.8 (10.4)	86.56 (15.8)	87.7 (10.0)
Response time, ms [b]	1152 (229)	1165 (185)	1090 (207)	1287 (262)	1083 (98)	1102 (147)	1216 (222)	1183 (154)

Except for sex, all data are presented as mean (standard deviation); **BD=** Bipolar Disorder; **BPRS=** Brief Psychiatric Rating Scale; **HDRS=** Hamilton Depression Rating Scale; **YMRS=**Young Mania Rating Scale. [a] Scores for BD patients are significantly greater than those for healthy individuals and unaffected first-degree relatives ($P < .02$); [b] Scores for BD patients are significantly greater than those for all other groups ($P < .02$).

Table 6-4 Effect of CACNA1C rs1006737 genotype (risk-allele A)

	Effect of Genotype		Effect of Group by Genotype					
	Risk Associated AA+AG	No-risk Associated GG	Risk Associated AA+AG			No-risk Associated GG		
	N = 57	N = 55	Unrelated Healthy Individuals N = 25	BD Patients N = 17	Healthy Relatives N = 15	Unrelated Healthy Controls N = 21	BD Patients N = 24	Healthy Relatives N =10
Demographic Variables								
Age(years)	37.9 (13.0)	37.5 (12.0)	40.1 (11.1)	44.4 (12.3)	36.53 (16.0)	38.1 (13.4)	44.1 (10.4)	36.0 (11.3)
Sex (Male/Female)	31/26	31/24	16/9	6/11	9/6	12/9	14/10	5/5
Clinical Features								
HDRS total score [a,b]	2.3 (2.4)	1.1 (1.5)	0.1 (0.5)	6.8 (6.5)	0.06 (0.2)	0.1 (0.5)	3.3 (3.8)	0.1 (0.3)
YMRS total score [a,b]	0.9 (1.3)	0.3 (1.0)	0.3 (0.6)	2.4 (3.5)	0.0 (0.0)	0.1(0.7)	0.8 (2.3)	0.0 (0.0)
BPRS total score [a,b]	24.9 (1.8)	24.8 (1.1)	24.4 (0.7)	26.4 (4.2)	24.1 (0.5)	24.2 (0.6)	26.3 (2.6)	24.1 (0.3)
Cognitive Performance								
IQ	113.1	113.7 (15.2)	101.5 (12.5)	118.7 (20.2)	119.5 (21.6)	111.8 (17.2)	116.9 (14.5)	112.5
Correctly identified faces, %	89.8 (11.1)	89.66 (10.5)	92.9 (6.5)	90.9 (7.9)	89.1 (9.6)	93.3 (6.6)	89.4 (8.8)	90.8 (6.5)
Response time, ms[b]	1159 (237)	1150 (185)	1125 (285)	1240 (249)	1088 (169)	1097 (128)	1265 (236)	1078 (121)

Except for sex, all data are presented as mean (standard deviation); **BD=** Bipolar Disorder; **BPRS=** Brief Psychiatric Rating Scale; **HDRS=** Hamilton Depression Rating Scale; **YMRS=** Young Mania Rating Scale. [a] Scores for BD patients are significantly greater than those for healthy individuals and unaffected first-degree relatives ($P < .02$); [b] Scores for BD patients are significantly greater than those for all other groups ($P < .02$).

Figure 6-1 Analysis of variance maps (axial sections presented in the standard space of Talairach and Tournoux with the z dimension shown numerically on the top left corner of each image) showing anatomical locations of the main effect of group (bipolarpatients, healthy relatives and healthy individuals) on activation in the affective relative to neutral facial expressions condition. Also shown are plots of the mean andstandard error of the weighted estimates of neural responses extracted from these areas for Healthy Controls (HC), Healthy Relatives (RELs),and Bipolar Disorder (BD) patients. IOG=Inferior Occipital Gyrus, AMY=Amygdala, FG=Fusiform Gyrus, VPFC=Ventral Prefrontal cortex.

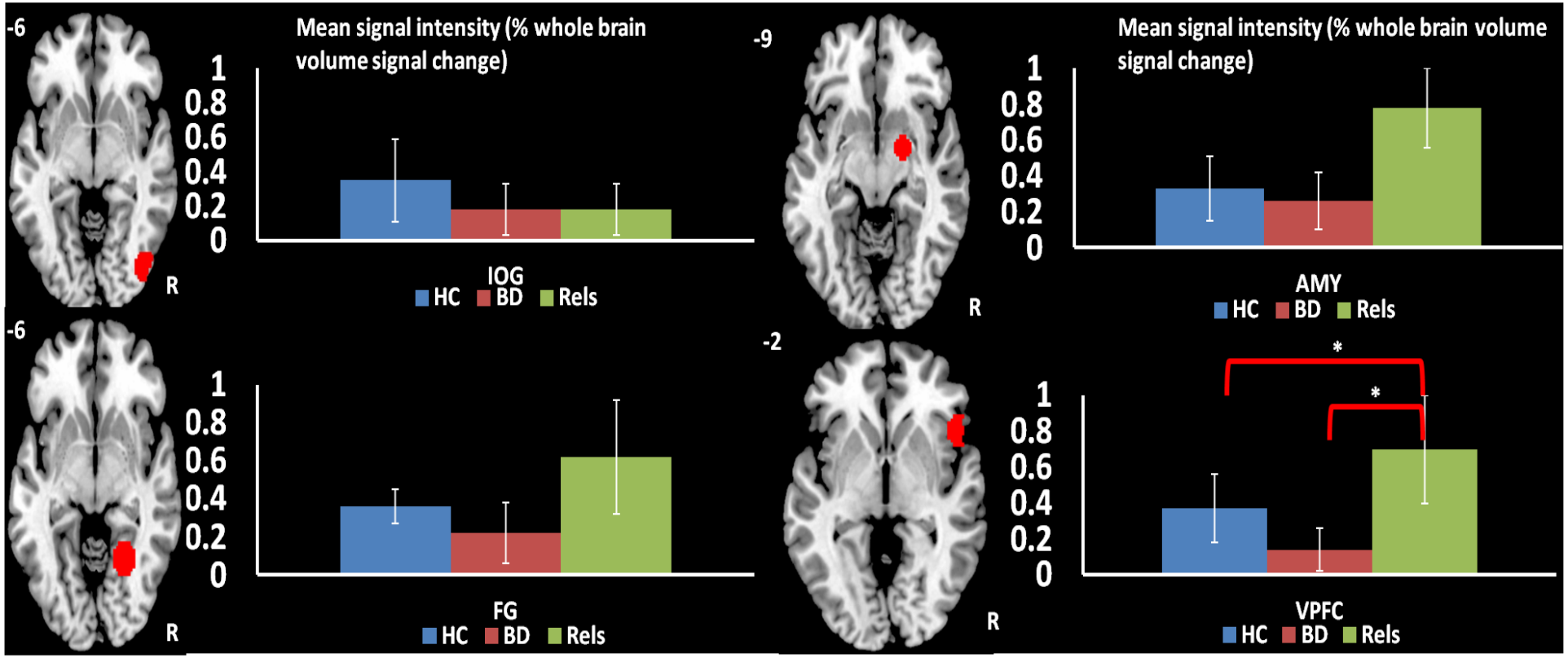


Figure 6-2 Main effect of genotype, ANK3 rs9810490, ANK3 rs10994336 and CACNA1C rs1006737 on facial affect labelling task in the affective relative to neutral facial expressions condition. Also shown are plots of the mean of the mean signal intensity (reported as a percentage of whole-brain volume signal change). IOG= Inferior Occipital Gyrus, AMY= Amygdala, FG= Fusiform Gyrus, VPFC= Ventral Prefrontal cortex.

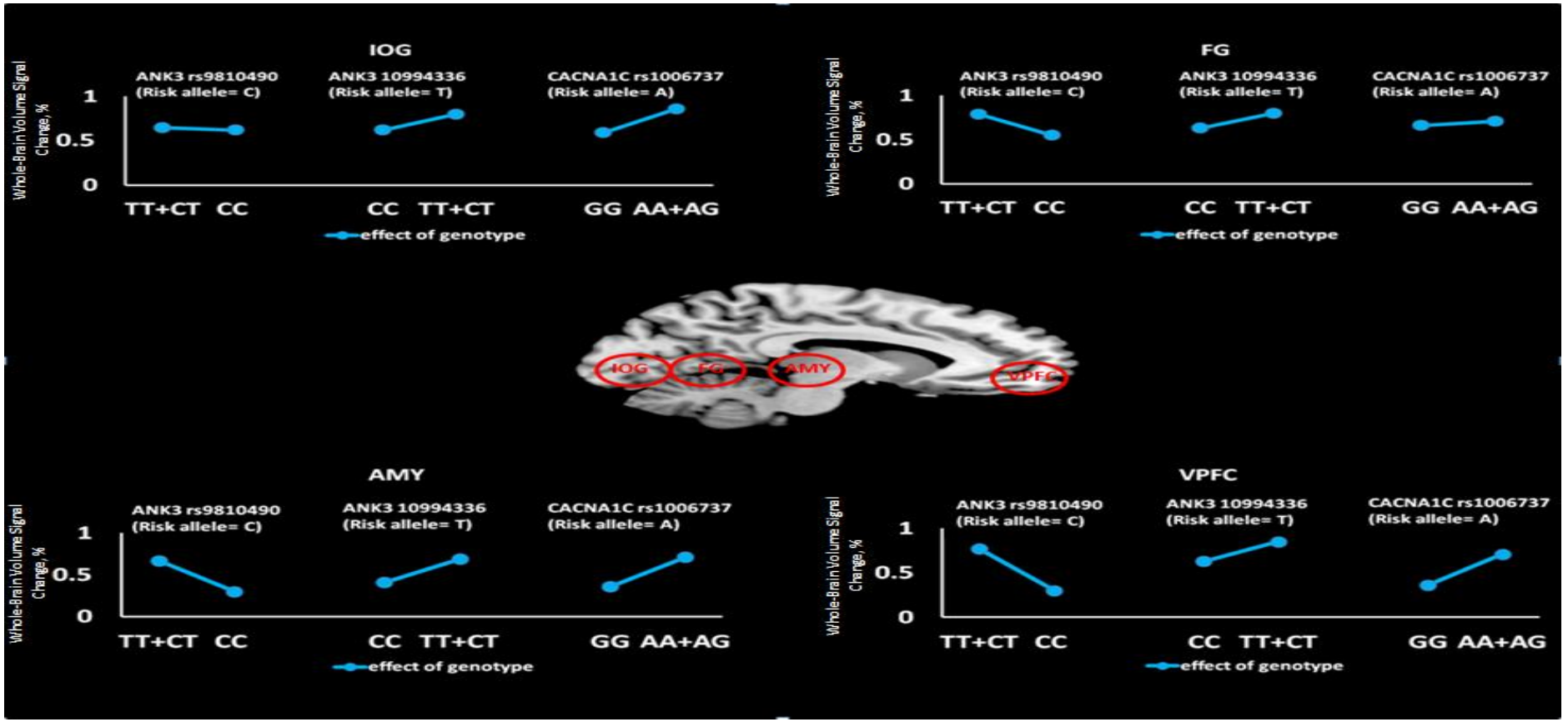
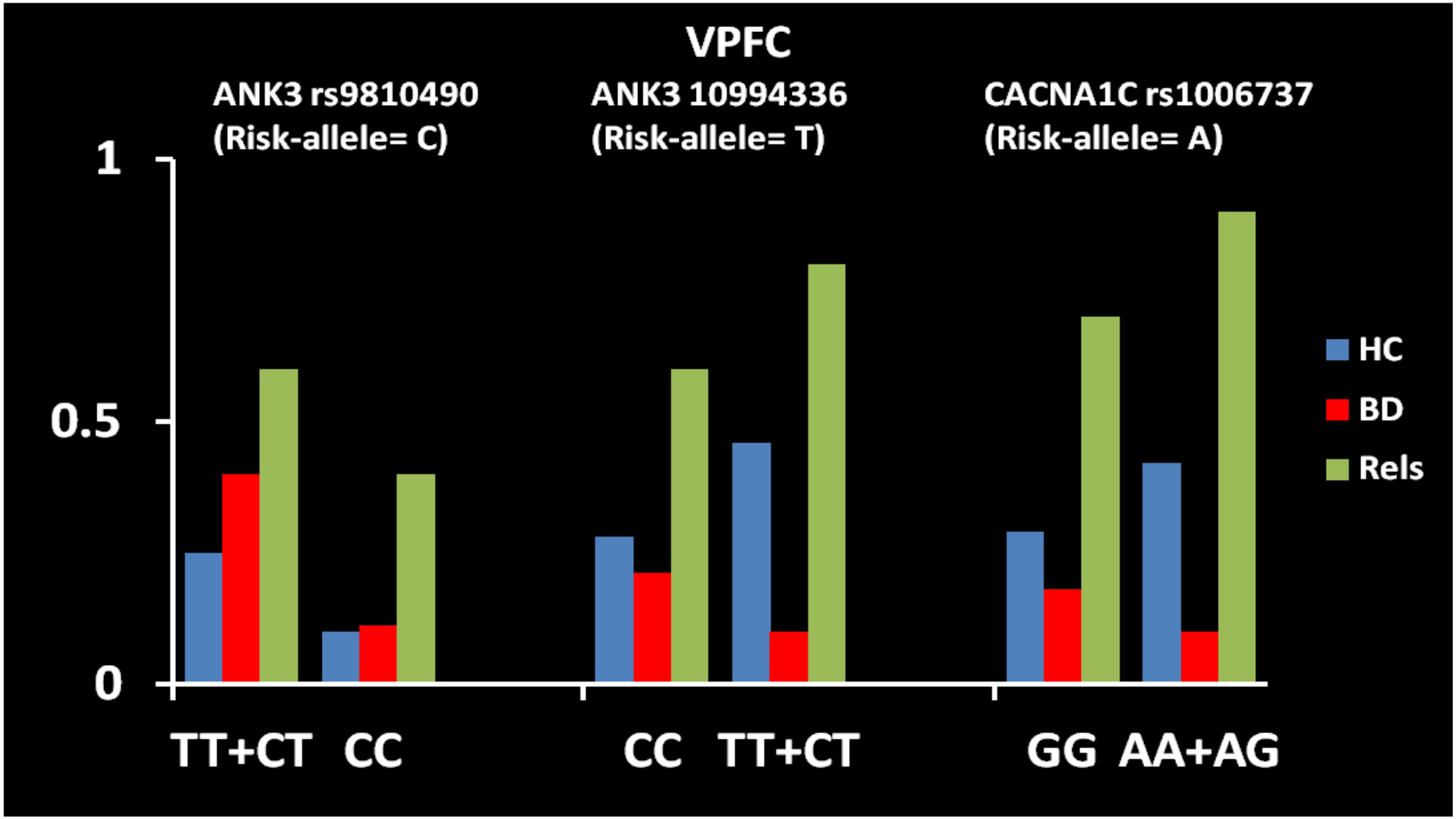


Figure 6-3 Group by ANK3 rs9810490, ANK3 rs10994336 and CACNA1C rs1006737 interaction on facial affect labelling task in the affective relative to neutral facial expressions condition in healthy controls (HC), healthy relatives (RELs),and Bipolar Disorder (BD) patients. Also shown are plots of the mean of the weighted estimates of neural responses extracted from the ventral prefrontal cortex (VPFC).



7. Discussion

7.1 Introduction

This chapter synthesizes the main findings and highlights, the strengths and limitations of the studies comprising chapter 3, chapter 5 and chapter 6 (**Chapter 3. Neural correlates of emotional processing in Bipolar Disorder: a voxel-based meta-analysis of functional magnetic resonance imaging studies; Chapter 5.The effect of ANK3 bipolar-risk polymorphisms on the working memory circuitry differs between loci and according to risk-status for bipolar disorder; and Chapter 6. FMRI investigation on the effect of CACNA1C and ANK3 risk genes on brain regions during processing of facial affect labelling task in Bipolar disorder, their healthy relatives and healthy individuals).** Finally,this chapter will conclude by discussing directions for future research.

7.2 Summary of the main findings

The main aims of this thesis were to (1) synthesise data from functional magnetic resonance imaging (fMRI) studies exploring brain regions underpinning facial affect processing in Bipolar Disorder (BD) by using meta-analytic techniques and (2) delineate the functional role of the CACNA1C and ANK3 risk genes on neural circuits relevant to BD. For the latter purpose, I used fMRI data to examine the effect of three genetic variants within the CACNA1C (rs1006737) and ANK3 (rs10994336 and rs9804190) risk genes on the neural correlates of (a) working memory and (b) facial affect labelling in BD patients, their healthy relatives and healthy individuals. In reference to the aims of this thesis, the following findings are presented:

1. The two meta-analyses included in chapter 3 aimed to show the common and distinct pattern of activation between BD, Major Depressive Disorder (MDD) and Schizophrenia (SZ) during processing of facial emotional tasks by using a meta-analytic technique: the Activation Likelihood Estimation (ALE). Briefly, this technique allows the data-mining and coordinate-based meta-analysis of fMRI results by employing a probabilistic approach (Laird et al., 2005). ALE estimates the probability that at least one activation focus from a collection of experiment truly lies at a specific voxels' location by use of Gaussian assumptions of spatial uncertainty (Eickhoff et al., 2009). The results of my two meta-

analyses not only elucidated the neuronal underpinnings of facial affect processing in BD but also delineated brain activation maps of MDD and SZ, disorders that overlap in terms of clinical symptoms (Fischer & Carpenter, 2009; Judd et al., 2002; Angst et al., 2010) and genetic risk factors (International Schizophrenia Consortium, 2009; Lichtenstein et al., 2009; McGuffin et al., 2003) with BD.

In BD, my results are in line with prior fMRI studies (Chen et al., 2011)and showed increased activation of emotional regions, including parahippocampus and amygdala, and decreased activation in the ventral prefrontal cortex (VPFC), area considered important for the cognitive control of emotions (Hariri et al., 2003). Moreover, it is noteworthy that the overengagment of the emotional systems was also found in MDD but not in SZ patients which showed, however, decreased activation.

In the direct comparison between BD and MDD I found that BD patients showed increased activation in the parahippocampus and amygdala compared to MDD and also decreased activation in the dorsal anterior cingulate.

Compared to SZ, BD patients showed increased activation of the pulvinar thalamus and decreased activation in the posterior associative cortices.

Taken together, these results underscore the engagement of common and disease-specific brain regions between BD, MDD and SZ suggesting that further examination of brain differences between these disorders may shed light to mechanisms differentiating BD from MDD and SZ.

2. (a) The fMRI study investigating the main effect of two risk single nucleotide polymorphisms (SNPs) located in the ANK3 gene, the rs10994336 and the rs9804190, and the group by genotype interaction for each SNPs during a working memory task, described in chapter 5, lead to two significant results:

i) For healthy individuals, I found that the two SNPs within the ANK3 risk gene affect different regions of the working memory circuitry. For the ANK3 rs10994336 I found decreased activation in the ventral visual cortex, including the middle and inferior temporal gyri, while for the ANK3 rs9804190 healthy individuals showed increased activation in the

prefrontal cortex, including the middle and inferior frontal gyri. These regions are both part of the superordinate cognitive control network (Niendam et al., 2012) but they subserve different functions.

The prefrontal cortex plays an important role in regulating executive function, such as working memory, cognitive flexibility and planning (Elliot et al., 2003). The increased activation observed in this region in carriers of the ANK3 rs9804190 risk-allele may be a reflection of the inefficient use of the prefrontal networks and therefore a deficit in the executive functions. In keeping with this interpretation is the evidence from a study by Roussos et al. (2012) which showed that healthy individuals carriers of the ANK3 rs9804190 risk-allele underperformed in several executive function tasks.

On the other hand, the ventral visual cortex is involved in the processing of visual stimuli, implicit or explicit, and the decreased activation in this area is in line with a previous study by Ruberto et al. (2011) which found that the ANK3 rs10994336 risk-allele was associated with decreased sensitivity in target detection.

ii) BD patients and their healthy relatives carriers of either the ANK3 rs10994336 or the ANK3 rs9804190 risk-allele showed increased activation in the ventral anterior cingulate cortex (ACC) compared to healthy individuals. Additionally, BD patients carriers of the ANK3 rs10994336 risk-allele also showed increased activation in the posterior cingulate cortex (PCC) compared to healthy individuals. Both regions have extensive reciprocal connections to multiple cortical and subcortcal regions (Hagmann et al., 2008; Paus et al., 2001) and they are key components of the default mode network (DMN), one of the most studied resting-state networks (Buckner et al., 2008; Raichle et al., 2001).

In healthy individuals, it has been consistently reported that the regions that are part of the DMN, including the ventral ACC and the PCC, are supposed to be deactivated during cognitive demanding tasks, and highly metabolically active during rest (Leech et al., 2011; 2014; Mazoyer et al., 2001). Therefore, the hyperactivation in the ventral ACC and PCC observed in this study in BD patients carriers of either risk-alleles support the evidence of a failure to deactivate the DMN network that therefore may interfere with the regions that are part of the cognitive control network. In keeping with this evidence are the results of an

fMRI study by Allin et al. (2010) that found a failure to deactivate the PCC in euthymic BD patients while performing a paced verbal fluency task. Finally, the same pattern of hyperactivation in the ventral ACC observed in healthy relatives carriers of the ANK3 rs10994336 risk-allelemay suggest a possible neurobiological marker for the familial risk of the BD condition.

2. (b) The fMRI study investigating the functional role of the three SNPs within the CACNA1C (rs1006737) and ANK3 (rs10994336 and rs9804190) genes on four predefined volume of interests (VOIs) during processing of facial affect labelling task reported in chapter 6 showed:

i) The three genetic polymorphisms regardless of group influenced key regions of the facial affect processing network with increased activation in the amygdala, fusiform gyrus, inferior occipital gyrus and VPFC for the CACNA1C rs1006737 and ANK3 rs10994336 risk-alleles and decreased activation in the same regions for the risk ANK3 rs9804190 allele. Together these findings add to the growing literature that showed stronger effects of these genes at the level of brain processing of emotional and cognitive information (Krug et al., 2010; Jogia et al., 2011; Bigos et al.,2010; Roussos et al., 2012).

ii) BD patients had decreased activation in the VPFC compared to healthy individuals and their healthy relatives. This result is in keeping with prior evidence which showed decreased VPFC activation in BD patients while processing emotional tasks (Altshuler et al., 2008; Foland et al., 2008; Jogia et al., 2008; Foland-Ross et al., 2012). Taken together these findings suggested that the dysfunction in the VPFC is a trait related feature of BD and represent a dysfunction of the cognitive control mechanism exerted by the VPFC over the amygdala (Jogia et al., 2008).

iii) In healthy relatives and healthy individuals the risk variants within the CACNA1C rs1006737 and ANK3 rs10994336 independently increased the activation in the VPFC. The implication of these findings has been linked to a compensatory mechanism of the top-down cognitive control system (Jogia et al., 2012) that it has been interpreted as the ability to effectively adapt to BD-related dysfunctions in the emotional processing network. In

contrast, the ANK3 rs9804190 risk-allele has been associated with decreased activation in the VPFC in the two groups.

7.3 Strengths and limitations

7.3.1 Strengths

This thesis has multiple strengths and advantages. Firstly, the two meta-analyses included in this thesis synthesized the results of fMRI studies conducted by independent researchers and therefore helped to identify neural patterns that are consistent and specific for BD, MDD and SZ. Consequently, they provided a clearer picture of the neural regions underpinning facial emotional processing in these psychiatric disorders.

Secondly, the two fMRI studies described in chapter 5 and chapter 6 of this thesis are among the first to report evidence about the different functional impact of genetic polymorphisms within the CACNA1C and ANK3 risk genes for BD during processing of cognitive and emotional tasks in BD patients, their healthy relatives and healthy individuals.

Thirdly, the participants that I used for all the fMRI analyses performed in this thesis were matched for age and sex in order to increased the homogeneity of the sample. Indeed, several studies in BD reported that both sex (Barret et al., 2008) and age (Weisenbach et al., 2014) have an effect on brain activation during processing of cognitive and emotional tasks.

Fourthly, the observed differences in brain activation during processing of cognitive (Chapter 5) and emotional (Chapter 6) tasks cannot be attributed to differences in accuracy or intellectual ability as the groups (healthy individuals, BD patients and healthy relatives) were comparable in terms of these measures.

Fifthly, BD patients included in this thesis were in euthymic state and therefore the results may underline the trait deficits of the disease rather than state related effects.

Finally, the results from the two imaging genetics studies included in this thesis suggested that by harnessing the information in the human genome is possible to uncover insights into the neurobiology of BD which in turn may point to new targets for the development of more effective treatments. This is true especially for the CACNA1C and ANK3 genes due to their

role in the regulation of the voltage-gated ion channels that, in the light of my findings, seem to be important for the proper development and function of neural circuits that regulate emotions and cognition, especially in BD patients. The voltage-gated ion channels are widely and selectively distributed in the brain and therefore it is not surprising that alterations in ion channel activity may be the underlying cause of the dysfunctions in the specific brain regions observed in my two imaging genetic studies. It has been shown that the activation of these channels influences neurotransmitter release, neuron excitability, gene transcription, and plasticity (Imbrici et al., 2013) and their activation or inhibition can alter the extracellular levels of several neurotransmitters, including dopamine, serotonin, glutamate, and GABA, which are notoriously implicated in psychiatric disorders (Aldana and Sitges, 2012; Mele et al., 2012). Therefore, discovering the important role of the CACNA1C and ANK3 genes at the level of brain function might be of interest in finding neurobiological mechanism underpinning the clinical symptoms observed and experienced in BD as well as in developing novel molecules acting as selective ion channels blockers and consequently in finding better treatments for BD.

7.3.2 Limitations

In this section I will report some limitations related to the results included in this thesis.

Firstly, the two meta-analyses included in chapter 3 present some methodological limitations that needed to be acknowledged: (a) I accepted the results of individual studies as reported and not weighted based on the threshold of significance used in each original study, (b) although most patients were remitted when scanned the level of patients' symptomatology varies in the studies included, (c) in the majority of the studies included, patients were medicated and were prescribed combinations of psychotropics and therefore the impact of medication on the results cannot be easily evaluated, (d) sex differences have not been explored, (e) most of the original studies examined multi-episode BD patients therefore the results of my meta-analyses could not be generalized to the initial stages of mood disorders, (f) it would be desirable to evaluate a range of study-wise factors that may influence both the nature and the topography of the results. These factors may include clinical information about age of onset or illness severity, task performance and details of data acquisition and analysis, (g) a further limitation is the variability in the types of tasks

used in individual source studies, which precludes separate analyses per task type because of small sets sizes, and finally the two meta-analyses provided an estimate of the probability of differential activation in brain regions when comparing diagnostic groups and not the mean activation difference in these regions. Therefore, traditional measures of heterogeneity and publication bias that are based on the effect size of group differences are not applicable.

Secondly, the relatively small sample size in the three groups included in chapter 5 and chapter 6 might have masked differences of small effect sizes. The small sample size together with the rarity of one of the alleles for each genotype, brought me to consider the heterozygotes together with the homozygotes of each genotype. Therefore, the results of my thesis did not show the effect of carriers of just one risk-allele (heterozygotes) on cognitive and emotional circuits.

Thirdly, the three investigated variants, the ANK3 rs9804190, the ANK3 rs10994336, and the CACNA1C rs1006737, are located in the intron of the genes and therefore not causing an amino acid substitution. As mentioned in chapter 6, it still not clear the effect of these non-coding regions on neural activation and therefore more studies are needed to fully characterize the mechanism by which alterations in CACNA1C and ANK3 expression result in brain function changes.

Fourthly, although the BD patients used in this thesis were in remission, they all have been treated with different psychotropics and therefore I cannot conclusively exclude a possible effect of medication. However, Keener et al. (2007) have reported that medicated BD patients showed similar pattern of activation compared to healthy individuals but more research exploring the effect of psychotropic medications in brain function and structure is needed.

Finally, I cannot exclude that the effects of the three three genetic variants observed in Chapter 5 and Chapter 6 could be due to other, yet unknown, genetic loci, in linkage disequilibrium (LD) with the ANK3 rs9804190, the ANK3 rs10994336 and CACNA1C rs1006737.

7.4 Future direction

The studies and the results discussed in this thesis point to new avenues of enquiry in BD. This section aims to highlight a variety of research directions that need to be pursued to advance our understanding on this disorder.

As mentioned in the limitations, in this thesis I was unable to investigate the effect of carriers of only one dysfunctional copy of each genetic variant (heterozygotes) within the CACNA1C and ANK3 genes on brain regional activation during cognitive and emotional tasks. Therefore, by increasing the sample size I will be able to divide the groups according to the three genotypes. This will be useful for extending my results and to provide more insight on the functional impact of these genes on brain activation.

Evidence from GWAS studies reported the involvement of several other risk genes for BD, including NCAN (Cichon et al., 2011) and ODZ4 (Green et al., 2013; Mühleisen et al., 2014). Therefore the examination of these genes on neural activation in BD patients could be a future research objective in order to identify the most relevant risk genes for BD. Moreover, it will be interesting to also explore the epistatic genetic effects of those genes to identify possible interactions that may contribute to the diathesis of BD.

The BD sample included in this thesis was in clinical remission and therefore I investigated the effect of ANK3 and CACNA1C risk genes on trait-related functional dysfunctions in BD. It would be interesting to explore the effect of these candidate genes in acute affective states (manic and depressive) to identify state related functional differences. It has been consistently shown that specific affective states might altered different brain regions while processing of cognitive or emotional tasks (Phillips et al., 2007; Townsend et al., 2012). However, evidence about the impact of specific genetic variants on BD in different affective states is still at its infancy.

GWAS studies reported a highly significant polygenic component of BD risk, involving up to thousand of common alleles with very small effect (Sklar et al., 2011; Ferreira et al., 2008; Mühleisen et al., 2014). Therefore, the scientific interest is now shifting towards the exploration of the aggregate effect of common genetic variants (alleles) in the context of a polygenic model, especially because psychiatric disorders are governed by pleiotropy and

polygenicity. The main reason is because with a polygenic approach, and therefore with the ensemble of genetic markers resulted significant in GWAS studies, it will be possible to increase the explanatory power of a given phenotype even in small samples (Dudbridge et al., 2013). The first successful application of polygenic score analysis to GWAS data was in schizophrenia (Purcell et al., 2009; Ripke et al., 2014). Notably, the recent study by Ripke et al. (2014) reported that the variance explained by the polygenic risk score (PRS) in schizophrenia is exponentially higher (18%) compared to the variance explain by a single SNP, e.g the CACNA1C gene (0.3%). Moreover, the PRS has also been used in correlation with neuroimaging measures. Specifically, two fMRI studies employed PRS for predicting brain regional dysfunctions, one in SZ (Walton et al., 2013) and the other in individuals with familial risk for a mood disorder (Whalley et al., 2012). Particularly, the study by Whalley et al. (2012) showed that increased polygenic risk-allele load for BD was associated with increased activation in limbic regions previously implicated in BD, including the ACC and amygdala, during a verbal fluency task. Finally, the clinical relevance of the polygenic scoring might be potentially striking especially with the discovery of even more genetic markers associated with a given disease. As the cost of sequencing decreases, the clinical utility of the DNA sequencing is currently undergoing intensive investigation as a tool for precise diagnosis, risk prediction, and therapeutic guidance (Biesecker et al., 2014). Specifically, some studies have already proven the clinical importance of the combination of established genetic risk variants in the discrimination of cases from controls, especially for prostate cancer (Aly et al., 2011; Zheng et al., 2008). Therefore, it is plausible that in the near future, the PRS may have the potential to improve the efficiency of health care system by helping to develop personalized treatments as well as preventive strategies also in common psychiatric diseases.

Finally, as described in chapter 5 and chapter 6, the two tasks employed in this thesis, the N-back and the facial affect labelling tasks, engage a large network of interconnected regions (Owen et al., 2005; Phillips et al., 2003). Therefore, it would be pertinent that future fMRI studies will focus their attention on investigating the association of genetic variants within the CACNA1C and ANK3 genes on multiple rather than isolated brain regions. With this regard, the dynamic casual modelling (DCM) method (Friston et al., 2003) might be useful for examining connectivity patterns of a putative distributed functional networks in BD.

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Appendices

Appendix 1: Original paper on:**" Risk and resilience in bipolar disorder: rationale and design of the Vulnerability to Bipolar Disorders Study (VIBES). Biochem Soc Trans 2009; 37: 1085-1089.**

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Risk and resilience in bipolar disorder: rationale and design of the Vulnerability to Bipolar Disorders Study (VIBES)

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Abstract

BD (bipolar disorder) is among the ten most significant causes of disability worldwide. Neuroscientists and clinicians have yet to meet the challenge of reducing this disability burden. The main obstacle to date has been our incomplete understanding of the pathophysiology of BD which thwarts primary prevention and early diagnosis and hinders effective treatment. There is a need to move beyond diagnostic approaches based purely on behavioural observation, as they lack reliability and biological validity. The present article reviews the evidence for cognitive, brain structural and functional correlates of genetic predisposition to BD and highlights biological markers of risk as well as factors that might protect against disease expression. It also outlines the rational and design of the Vulnerability to Bipolar Disorders Study (VIBES), which exemplifies a promising approach to delineating biological mechanisms mediating risk, resilience and disease expression in BD.

Introduction

BD (bipolar disorder) is characterized by repeated episodes mania or hypomania intermingled with episodes of depression [1]. It is now recognized that most patients will have a recurrent illness associated with significant disability in terms of social, marital or occupational function. It is therefore not surprising that the World Health Organization has listed BD among the 30 leading causes of global burden of disease [2].

Defining the neural circuitry involved in mood regulation is fundamental to understanding the pathophysiology of BD. Several brain regions are involved in emotional processing and in the integration of emotion with cognition and visceral functions. These include the PFC (prefrontal cortex), the ACC (anterior cingulate cortex), the amygdala, the parahippocampal gyrus and the hippocampus. These regions are heavily interconnected and are also connected with other brain structures, particularly the thalamus, hypothalamus and striatum.

The extended phenotype of BD

It is now widely accepted that BD is associate with changes in cognition, brain structure and function even during periods of complete illness remission.

Key words: bipolar disorder, brain functional change, depression, functional magnetic resonance imaging (fMRI), Vulnerability to Bipolar Disorders Study (VIBES).

Abbreviations used: ACC, anterior cingulate cortex; BD, bipolar disorder; BDI, BD type I; DLPFC, dorsolateral prefrontal cortex; fMRI, functional magnetic resonance imaging; HDRS, Hamilton Depression Rating Scale; MDD, major depressive disorder; MRI, magnetic resonance imaging; PFC, prefrontal cortex; rCBF, regional cerebral blood flow; VIBES, Vulnerability to Bipolar Disorders Study; VPFC, ventral prefrontal cortex; YMRS, Young Mania Rating Scale.

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Cognitive changes

The pattern of trait cognitive changes in BD is still under investigation, since small sample sizes and large variability in the tests used to assess cognition have resulted in significant heterogeneity between studies. Several reviews and meta-analyses have attempted to overcome these problems [3–5]. The emerging consensus is that BD patients are impaired in most cognitive domains during acute mood episodes, whereas abnormalities in memory and aspects of executive control of attention, response initiation and inhibition may persist during inter-episode intervals.

Brain structural changes

Kempton et al. [6] have published the most recent meta-analysis of available brain structural studies ($n = 141$) comparing BD patients with healthy individuals. They found lateral ventricular volume to be significantly increased (+17%) in BD patients, whereas differences in other brain regions, including total grey and total white matter, prefrontal and temporal volume, the amygdala and hippocampus, basal ganglia and cerebellum (vermis and hemispheres), were of small to modest effect (effect size less than 0.5). Neither demographic nor illness characteristics influenced these results.

Brain functional changes

During depressive episodes, a decrease in the activity of the DLPFC (dorsolateral prefrontal cortex) and increases in the amygdala [7,8] have been reported in resting state functional imaging studies. Manic states have been associated with decreased activity in the VPFC (ventral prefrontal cortex) [9] and increased activity in the ACC [10].

In contrast with resting state studies, fMRI (functional magnetic resonance imaging) investigation of brain function

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employs a variety of activation paradigms. Trait-related decreases in brain activation within the left VPFC (Brodmann area; BA47) have been reported in BD patients compared with healthy participants [10] during response-inhibition paradigms; conversely, abnormalities within the DLPFC, suggestive of decreased engagement, have been reported in fMRI studies of working memory [11–14].

The facial affect discrimination task has been widely used to probe the neural correlates of emotional processing in BD. Yurgelun-Todd et al. [15] were among the first to use the facial affect discrimination paradigm to compare brain activation with fearful and happy expressions in BD patients and controls. BD patients showed reduced activation in the DLPFC and increased in the amygdala in response to fearful facial affect, a pattern that has been confirmed in subsequent studies.

The phenotypic spectrum of individuals with genetic predisposition to BD

Genetic factors are important in the aetiology of BD [16]. However, the spectrum of behavioural and psychiatric abnormalities associated with the predisposition to BD is very wide.

Clinical phenotypes

First-degree relatives of probands with BDI (BD type I) are at increased risk of not only BD, but also MDD (major depressive disorder); morbidity risk estimates range between 1.1 and 4.9% for BDI and 5.8 and 14% for MDD [17]. There is little evidence of specificity in familial aggregation for the clinical phenotype of BD, suggesting that the relationship between genetic predisposition and clinical phenotypes is complex even when heritability is very high, as is the case with BD [16].

Cognitive phenotypes

The high rates of psychiatric morbidity in first-degree relatives of BD have prompted researchers to examine potential expressions of genetic predisposition to BD in terms of cognitive function. There have been four recent reviews and meta-analyses of cognitive studies in unaffected first-degree relatives of BD patients [5,18–20]. Arts et al. [5] identified verbal learning and verbal working memory impairment as the most consistent deficit in relatives of BD patients; the effect size of the difference between unaffected relatives and healthy individuals was the largest (approx. 0.8) for these measures. In all other domains, first-degree relatives showed evidence of subtle cognitive changes with a much smaller effect size (<0.5). Bora et al. [20] additionally reported that both patients and relatives shared deficits in response inhibition, set shifting and sustained attention; the difference was quantitative with medium to large effect sizes seen in patients, whereas small to medium effect sizes were observed in relatives.

Surprisingly in BD, only one study to date has compared the cognitive profile of individuals with BD with that of patients with other affective disorders from bipolar pedigrees. Savitz et al. [21] examined the effect of diagnosis on verbal fluency, memory, interference and abstraction/set shifting in 230 relatives from 47 families; these comprised 49 individuals with BDI, 19 with BD type II, 77 with depressive illness, 20 with miscellaneous diagnosis and a group of 65 psychiatrically healthy relatives. They reported that BDI patients performed less well than their MDD counterparts as well as all of their other relatives in memory (recall and recognition) and learning.

Structural phenotypes

Several neuroimaging studies have attempted to define the pattern of brain structural changes associated with genetic predisposition in BD, but the results have generally been inconsistent.

McDonald et al. [22] collected structural MRI (magnetic resonance imaging) data from 37 patients with BD and 50 first-degree relatives. Through the use of a genetic liability scale, they inferred that genetic risk of BD was negatively correlated with grey matter volume in the right medial frontal gyrus, anterior cingulate gyrus, caudate nucleus and anterior putamen (BA9, BA11, BA24, BA25 and BA32). Despite using a similar study design, McIntosh et al. [23] failed to replicate these findings. They employed optimized voxel-based morphometry to investigate the effects of genetic liability to BD on white and grey matter volume in 22 well relatives from families affected by BD alone, i.e. unaffected relatives with at least two first- or second-degree relatives with BD. They did not show any significant relationship between a genetic liability to bipolar disorder and either white or grey matter volume. Both research groups conducted further analyses on their samples. McDonald et al. [24] used a hypothesis-based region of interest analysis to assess ventricular and hippocampal volumes; they did not find significant differences between BD relatives and controls, although relatives showed a 2.3% increase in total cerebral volume. Similarly, McIntosh et al. [25] carried out an alternative voxel-based morphometry study on their sample of 22 unaffected individuals with a family history BD. Following small volume correction, they found decreased grey matter density in the bilateral anterior thalamus and in the body of caudate on the left. These findings contrast with the report by Noga et al. [26] of increased caudate volume in healthy co-twins of BD patients compared with controls. Finally, no volume changes in the subgenual anterior cingulate cortex, but larger hippocampal volume were reported by Hajek et al. [27] and Ladoucer et al. [28] respectively in two independent region-of-interest studies of unaffected offspring of BD patients. Taken together, these studies suggest that, in individuals at high risk of BD, brain structural changes are probably of small effect size, whereas the direction of change in cortical and subcortical regions remains unclear.

Brain functional phenotypes

Krüger et al. [29] used $H_2{}^{15}O$ positron emission tomography to study a cohort of nine lithium-responsive BD patients and nine healthy siblings. They collected rCBF (regional cerebral blood flow) data during baseline euthymia and during induction of transient sadness using a short autobiographical script, which related to a sad life event. During induced

sadness, patients and their siblings showed rCBF increase in the ACC (BA24), the anterior insula, the premotor cortex (BA4/6) and cerebellum. Both groups showed rCBF decrease in orbitofrontal cortex (BA11) and inferior temporal cortex (BA20/21). However, rCBF in the medial PFC (BA10) was decreased in BD patients and increased in their siblings. Krüger et al. [29] postulate that increased rCBF in siblings represented a compensatory response in an at-risk group, as this pattern was not seen previously in healthy subjects without depression risk factors [30].

Drapier et al. [13] focused on working memory function. They used fMRI to compare patterns of brain activation between 20 BD patients, 20 of their unaffected first-degree relatives and 20 healthy participants while performing the N-back verbal memory task. During this task, participants are presented with a series of stimuli (verbal or non-verbal) and are asked to indicate whether the stimulus they are currently viewing matches the one they saw in the previous one, two or three trials. This task allows for group comparisons within a single condition as well as for the examination of group differences in response to increasing working memory load across conditions. Patients' performance in the 2-back and 3-back conditions were worse than their relatives and controls. Significant group differences were observed in a left ventral cluster extending from the frontopolar to VLPFC (BA10/47). Relatives showed greater activation in these regions than the other two groups, particularly in the 2-back condition. The authors suggested that the increased activation within PFC regions in the relatives group reflected cortical inefficiency (i.e. recruitment of wider networks to maintain adequate performance).

It would appear that increased prefrontal activation is associated with genetic risk of BD, but not with disease expression. The biological meaning of this finding is still unclear, but it is likely to be associated with aspects of neural functioning that protect against or compensate for disease expression.

VIBES (Vulnerability to Bipolar Disorders Study): rationale, design and sample

Although BD is highly heritable, individuals with genetic predisposition to BD are also at increased risk of a wide spectrum of phenotypes, including a range of psychiatric disorders as well as cognitive and brain functional and structural changes. The mechanisms involved in translating familial risk to BD into specific phenotypes remain largely unknown. The core aims of VIBES are to address the following questions: in individuals at high familial risk of BD (i) what are the genetically mediated traits shared with patients and by at-risk, but non-symptomatic, relatives, and (ii) can we identify disease-specific traits that differentiate BD patients from their relatives with other diagnoses such as MDD? Each of the study modules focuses on characterizing the cognitive, structural or functional brain-related changes associated with familial predisposition to BD. However, the set of biomarkers that best distinguishes between predisposing, protective and disease-related mechanisms will probably include measures from each of these modalities. Hence, we use bioinformatic techniques to determine which variables are uniquely sensitive to the expression of different phenotypes among individuals with a genetic predisposition to BD. This set of biomarkers should provide tools for subsequent neuropsychiatric, pharmacological and genetic research.

Table 1 | **VIBES cognitive test battery**

Test	Neuropsychological domain
Wechsler Adult Intelligence Scale-Revised	Full-scale IQ
Hayling Sentence Completion Test (HSCT)	Response inhibition
Wisconsin Card Sorting Test (WCST)	Rule discovery and perseveration
Continuous Performance Task (CPT)	Sustained attention
Wechsler Memory Scale-III (WMS-III)	Auditory and visual immediate and delayed auditory recognition, delayed and working memory
Stroop Colour Word Test (SCWT)	Interference
N-Back sequential letter working memory task	Working memory
Iowa Gambling Task (IGT)	Emotional learning

Eligibility criteria

Inclusion criteria for BD patients are: (i) being aged between 17 and 65 years, (ii) fulfilling Diagnostic and Statistical Manual of Mental Disorders, 4th edition, revised (DSM-IV) [1] criteria for BDI, (iii) having at least one first-degree relative unaffected by BD, and (iv) no family history (up to second degree) of schizophrenia or schizophrenia spectrum disorders.

Their siblings and offspring were eligible to participate, with the patients' consent, if (i) aged 17–65, and (ii) had no personal history of bipolar spectrum disorders.

Inclusion criteria for control participant are: (i) being aged 17–65 years, and (ii) having no personal or family history of any Axis I or II DSM-IV disorder. Healthy volunteers were selected so that they matched both patients and relatives in age, gender and level of education.

Exclusion criteria for the entire sample are: (i) head trauma resulting in loss of consciousness, (ii) personal history of neurological or medical disorders, (iii) family history of hereditary neurological disorders, and (iv) fulfilling DSM-IV criteria for lifetime drug- or alcohol-dependence and drug or alcohol abuse in the preceding 6 months.

Clinical assessments

Diagnostic assessments for all participants were conducted by trained psychiatrists using the Structured Clinical Interview for DSM-IV for Axis I diagnoses. Inter-rater reliability was $\kappa > 0.92$. Where applicable, further information was collected from medical notes. Family history of psychiatric disorders

Table 2 | VIBES sample: clinical and demographic characteristics
BPRS, Brief Psychiatric Rating Scale.

	BD patients ($n = 47$)	Relatives with Axis I diagnoses ($n = 33$)	Healthy relatives ($n = 48$)	Controls ($n = 71$)
Age	46.2 (11.5)	31.4 (11.1)	36.5 (13.8)	39.8 (15.3)
Gender numbers (female/male)	26/21	22/11	19/23	35/36
Age of onset	25.5 (8.2)	20.6 (10.3)	N/A	N/A
Illness duration	20.0 (9.1)	3.5 (4.5)	N/A	N/A
Psychosis	32 (68.1%)	0	N/A	N/A
HDRS	3.0 (3.9)	1.3 (2.0)	0.00 (0.3)	0.2 (0.5)
YMRS	1.3 (2.9)	0.3 (1.0)	0.00 (0.0)	0.2 (0.5)
BPRS	26.9 (3.5)	25.4 (2.2)	24.0 (0.3)	24.4 (0.6)

was assessed using the Family Interview for Genetic Studies. Psychopathology was rated using the HDRS (Hamilton Depression Rating Scale) [31], the YMRS (Young Mania Rating Scale) [32] and the BPRS (Brief Psychiatric Rating Scale) [33]. Before assessment, participation patients' psychopathology was assessed weekly over a minimum period of 1 month to ensure that they: (i) fulfilled DSM-IV criteria for remission requiring a minimum period of 6 months of remission since the last syndromal episode, (ii) scored below 7 in the HDRS and YMRS, and (iii) had remained on the same type and dose of medication for a minimum period of 6 months.

Neuropsychological assessment

VIBES includes a comprehensive cognitive battery of tests aiming to evaluate domains of cognitive function as shown in Table 1.

Neuroimaging

Data for the structural and functional MRI module were acquired on a 1.5T General Electric Signa System scanner based at the Maudsley Hospital. The fMRI module comprised four paradigms aiming to examine working memory (N-back sequential-letter working memory task), response inhibition (Stroop Colour Word Test), decision-making (Iowa Gambling Task) and emotional processing (Facial Affect Discrimination Task).

Sample

A total of 92 BD probands from an equal number of families were screened by telephone interview for eligibility by a trained psychiatrist. Families from 53 BD probands were enrolled into the study. From the remaining 39 families that were initially screened, 24 families were excluded because the majority of the relatives refused to participate, also 15 families had no suitable relatives (either unavailable or not eligible). The 53 families that agreed to participate comprised 47 BDI patients (six patients did not achieve remission criteria throughout the study period) and 81 first-degree relatives. Key demographic and clinical details of the sample are shown in Table 2.

Conclusions

Our understanding of BD, like that of most psychiatric illnesses, lags behind that of other brain disorders in part because of the use of heterogeneous diagnostic constructs and the lack of clear biomarkers. Better delineation of the pathophysiology of BD should improve diagnostic classification including premorbid identification of subjects at risk of disease expression. It could thus facilitate the development of innovative new pharmacological and psychosocial treatments and clarify the role of potential risk conferring genes for BD.

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Appendix 2: Original paper on:**"Common and distinct neural correlates of emotional processing in Bipolar Disorder and Major Depressive Disorder: A voxel-based meta-analysis of functional magnetic resonance imaging studies." European Neuropsychopharmacology2012 Feb; 22: 100-113.**

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Common and distinct neural correlates of emotional processing in Bipolar Disorder and Major Depressive Disorder: A voxel-based meta-analysis of functional magnetic resonance imaging studies

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Abstract

Neuroimaging studies have consistently shown functional brain abnormalities in patients with Bipolar Disorder (BD) and Major Depressive Disorder (MDD). However, the extent to which these two disorders are associated with similar or distinct neural changes remains unclear. We conducted a systematic review of functional magnetic resonance imaging studies comparing BD and MDD patients to healthy participants using facial affect processing paradigms. Relevant spatial coordinates from twenty original studies were subjected to quantitative Activation Likelihood Estimation meta-analyses based on 168 BD and 189 MDD patients and 344 healthy

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controls. We identified common and distinct patterns of neural engagement for BD and MDD within the facial affect processing network. Both disorders were associated with increased engagement of limbic regions. Diagnosis-specific differences were observed in cortical, thalamic and striatal regions. Decreased ventrolateral prefrontal cortical engagement was associated with BD while relative hypoactivation of the sensorimotor cortices was seen in MDD. Increased responsiveness in the thalamus and basal ganglia were associated with BD. These findings were modulated by stimulus valence. These data suggest that whereas limbic overactivation is reported consistently in patients with mood disorders, future research should consider the relevance of a wider network of regions in formulating conceptual models of BD and MDD.

1. Introduction

Bipolar Disorder (BD) and Major Depressive Disorder (MDD) are amongst the leading causes of disability worldwide (Murray and Lopez, 1997). Although syndromal mania is unique to BD, both disorders present with recurrent depressive episodes as well as similar subsyndromal affective symptoms (Judd et al., 2002, 2003; Angst et al., 2010). Evidence from genetic studies also suggests both distinct and common contributions to their aetiology (McGuffin et al., 2003).

Current neurobiological models propose that mood disorders arise from disruption in prefrontal, limbic and subcortical regions (particularly the amygdala/hippocampus, and striatum) that support the adaptive regulation of affect (Savitz and Drevets, 2009). Within this general framework, much research effort in neuroimaging is directed towards identifying overlapping and diagnosis-specific brain abnormalities for BD and MDD. Several reviews and meta-analysis have attempted to summarise and synthesise the available evidence (Savitz and Drevets, 2009; Konarski et al., 2008). In the most recent quantitative meta-analysis (Kempton et al., 2011), we showed that volume reductions in the basal ganglia and hippocampus appear specific to MDD patients and differentiated MDD from BD. We now focus on the neural correlates of emotional processing in BD and MDD, which may relate more directly to the core abnormalities underpinning mood disorders. Our understanding of the neural circuitry involved in emotional processing in healthy individuals is mostly based on studies using facial affect as a probe (Phan et al., 2002; Murphy et al., 2003; Fusar-Poli et al., 2009; Vytal and Hamann, 2010). Facial affect processing is mediated by a distributed neural network that encompasses visual, limbic, and prefrontal regions (Phan et al., 2002; Murphy et al., 2003; Fusar-Poli et al., 2009; Vytal and Hamann, 2010). This network shows significant overlap with that implicated in mood disorders (Savitz and Drevets, 2009). We used Activation Likelihood Estimation (ALE) (Turkeltaub et al., 2002; Laird et al., 2005; Eickhoff et al., 2009), a quantitative meta-analytic approach which allows integration of neuroimaging results across studies, to investigate the neural correlates of facial affect processing in BD and MDD.

The main goals are threefold. First, to consolidate neuroimaging findings associated with emotional processing in patients with BD or MDD and to examine whether meta-analytic synthesis of this empirical evidence aligns with current theoretical models of mood disorders (Cerullo et al., 2009; Savitz and Drevets, 2009). Second, to determine whether stimulus valence modulates disease-related activity within the face processing network based on findings that neural activity and connectivity may differ between BD and MDD in response to positive and negative stimuli (Almeida et al., 2009; Almeida et al., 2010). Third, to identify common and distinct brain functional changes in BD and MDD.

2. Method

2.1. Data sources and inclusion criteria

Studies investigating facial affect processing in either BD or MDD patients were identified through a comprehensive MEDLINE, EMBASE and PsycINFO search of the English-language literature covering publications between January 2000 and December 2010. The search keywords were "mania", "depression", "bipolar disorder", "major depressive disorder" and "facial affect", "emotional processing", "fMRI" and their combinations as well as terms specifying individual facial affect (fear, happiness, sadness, anger and disgust). Additional articles were identified through the reference lists of these papers.

Studies were included if they (a) reported comparisons between patients with BD or MDD with healthy controls (b) employed functional magnetic resonance imaging (fMRI) (c) assessed brain activation by using human facial identities (d) used image subtraction methodology to identify foci of task-related neural changes contrasting an active (emotional faces) and control (neutral faces or shapes) condition, and (e) reported their results in standard stereotactic coordinates (either Talairach or Montreal Neurological Institute [MNI] space).

We excluded studies that (a) used facial affect stimuli to investigate processes not directly involved in emotional processing (e.g. memory, attention), (b) involved non-facial identities such as emotional pictures, (c) grouped together stimuli displaying positive and negative facial affect, and (d) used the same patient sample. The threshold of statistical inference varied but we accepted the results reported as significant based on the criteria of the primary studies.

2.2. Quantitative meta-analytical voxel-based procedure

We investigated facial affect processing in BD and MDD by focusing on the contrast between facial affect and control conditions using Activation Likelihood Estimation (ALE) implemented in GingerALE 2.0.4 (http://brainmap.org/Ale). This ALE version uses a random effect model and weighting for sample size of the original studies (Eickhoff et al., 2009). Coordinates of the foci of activation reported in the primary literature were transformed into Talairach space using the Lancaster transform (icbm2tal tool) in GingerALE. For each study, peaks were modelled as the centre of a 3D Gaussian distribution and a modelled activation (MA) map was then

Table 1 Studies included in the meta-analyses (alphabetical order).

Study	Participants (Male/female)	Age Mean, years (standard deviation)	Design	Contrast used in meta-analysis
Almeida et al. (2010)	BD (remitted) 15 (5/10) BD (depressed) 15 (1/14) 15 MDD (2/13) 15 HC (3/12)	BD (remitted): 33.2 (7.8) BD (depressed): 36.5 (11.8) MDD: 32.7 (9.8) HC: 32.6 (8)	Explicit facial affect labelling Event related	Sad>neutral
Altshuler et al. (2008)	11 BD(5/6) 17 HC (9/8)	BD: 32 (7.3) HC: 29.5 (6.6)	Explicit facial affect matching Block	Fear and angry> shapes
Blumberg et al. (2005)	17 BD (10/7) 17HC (7/10)	BD: 40 (12.3) HC: 33.2 (10.8)	Implicit facial affect processing Block	Happy>fixation cross
Chen et al. (2006)	8 BD (depressed) (5/3) 8 BD (manic) (8/0) 8 HC (2/6)	BD (depressed): 41.8 (12) BD (manic): 39 (13.4) HC: 38.7 (12.5)	Explicit and implicit facial affect recognition Event related	Fear>neutral (explicit) Happy>neutral (explicit)
Foland et al. (2008)	9 BD (3/6) 9 HC (3/6)	BD: 34.6 (8.0) HC: 30.4 (7.6)	Explicit facial affect matching Block	Fear and anger> shapes
Fu et al. (2007)[1]	19 MDD (6/13) 19 HC (8/11)	MDD:43.2 HC:42.8	Implicit Facial Affect Processing Event-Related	Happy>fixation
Gotlib et al. (2005)	18 MDD (5/13) 18 HC (5/13)	MDD: 35.2 HC:30.8	Implicit facial affect processing Block	Happy>neutral Sad>neutral
Hassel et al. (2008)	19 BD (10/9) 24 HC (11/13)	BD: 32.47 HC: 27.78	Implicit facial affect processing Event-related	Happy>neutral
Jogia et al. (2008)[1]	12 BD (5/7) 12 HC (5/7)	BD: 42.1 (11.8) HC: 41.8 (10.9)	Explicit facial affect recognition Event-related	Sad>neutral
Killgore et al.(2008)	14 BD (11/3) 13 HC (12/1)	BD: 28.1 (11.2) HC: 25.5 (4.7)	Implicit facial affect processing Block	Fear>fixation
Lawrence et al. (2004)	20 BD 9 MDD 11 HC 60% male	Overall mean 41 (11)	Implicit facial affect processing Event-related	Fear>neutral Happy>neutral Sad>neutral
Lee et al. (2008)	21 MDD (3/18) 15 HC (2/13)	MDD: 46.8 (9.1) HC: 48.7 (3.5)	Explicit facial affect rating block	Sad>fixation
Lennox et al. (2004)	10 BD (8/2) 12 HC (6/6)	BD: 37.3 (12.8) HC: 32.6 (10.7)	Explicit facial affect rating Event-related	Sad>neutral
Malhi et al. (2007)	10 BD (0/10) 10 HC (0/10)	BD: 33.5 (8.7) HC: 32.4 (6.4)	Explicit facial affect recognition Event-related	Fear>neutral
Norbury et al. (2010)	16 MDD (7/9) 21 HC (11/10)	MDD: 36.2 HC: 32.3	Explicit facial affect matching Block	Fear>Shapes
Suslow et al. (2010)	30 MDD (17/13) 26 HC (12/14)	MDD: 38.8 (11.4) HC: 36.2 (13.4)	Implicit facial affect processing Event-related	Sad>neutral
Scheuerecker et al. (2010)	13 MDD (10/3) 15 HC (10/5)	MDD: 37.9 (10.1) HC: 35.5 (10.9)	Implicit and explicit facial affect matching Block	Sad and angry> shapes (implicit)
Thomas et al. (2010)	30 (remitted) MDD (9/21) 35 HC (12/23)	MDD: 32.8 (10.4) HC: 31.7 (9.8)	Implicit facial affect processing Block	Sad>neutral Fear>neutral
Townsend et al. (2010)	15 MDD (9/6) 15 HC (9/6)	MDD: 45.6 (11.2) HC: 44.8 (11.7)	Explicit facial affect matching Block	Sad and angry> shapes
Van Wingen et al. (2010)	18 MDD (first episode) (7/11) 21 (remitted) MDD (4/17) 30 HC (13/17)	MDD (first episode): 33.3 (11.7) MDD (recovered): 34.5 (11.4) HC: 35 (12)	Explicit facial affect matching Block	Fear and angry> shapes

BD=Bipolar Disorder; MDD=Major Depressive Disorder; HC=Healthy Controls; 1=only baseline data used; 2=study also included 20 first degree relatives.

Table 2 Clinical description of MDD and BD patients includes in the meta-analyses.

Study	Psychopathology measures Mean, total score (standard deviation)	Duration of illness Mean, years (standard deviation)	Psychotropic medication (number of patients)	Diagnostic instrument	Comorbidity (number of patients with comorbidity/ total sample)	First or multiple episodes
Almeida et al. (2010)	YMRS score BD (depressed): 21.5 (6.4) BD (remitted): 1.4 (1.1) MDD: 24.4 (6.1)	BD (depressed): 14.2 (9.8) BD (remitted): 14.6 (5.4) MDD: 13.6 (9.8)	Unspecified	SCID	9/30 BD substance abuse 3/15 MDD substance abuse	All patients had experienced at least two episodes of illness in the last 4 years
Altshuler et al. (2008)	YMRS score BD:2.9 (1.9) HDRS score BD: 20.8 (3.3)	Unspecified	Medication free (2), Li (1), anticonvulsants (7), atypical antipsychotics (2), antidepressants (3)	SCID	No comorbid axis I and II disorders	Unspecified
Blumberg et al. (2005)	HDRS ARSM Unspecified scores	Unspecified	Medication free (5), Li (4), anticonvulsants (8), atypical antipsychotics (1), antidepressants (3)	Unspecified	9/12 lifetime history of alcohol dependence; >1.5 years in remission	Unspecified
Chen et al. (2006)	YMRS score BD(manic): 24.1 (8.2) BD(depressed): 0.4 (0.5) HDRS score BD (manic): 2 (2.9) BD (depressed): 18.3(6.4)	Unspecified	Li (11), anticonvulsants (9), typical (2) and atypical antipsychotics (3), antidepressants (2)	Unspecified	No comorbid axis I and II disorders	All patients had at least 2 previous episodes of mania and depression
Foland et al. (2008)	YMRS score BD: 15.1 (3.7) HDRS scores BD: 9.1 (5.3)	14.8 (5.1)	Li (20), anticonvulsants (6), atypical antipsychotics (1)	SCID	No comorbid axis I disorders	Mean manic episodes 4.2 (2)
Fu et al. (2007)	HDRS score MDD: 21.1 (2.3)	Unspecified	Medication free at baseline	SCID	No comorbid axis I and II disorders	Multi-episode unspecified
Gotlib et al. (2005)	BDI score MDD: 24.6 (8.3)	Unspecified	Antidepressants (8)	SCID	No comorbid axis I and II disorders	Multi-episode unspecified
Hassel et al. (2008)	YMRS score BD:<10 HDRS scores BD: <7	BD: 10.6 (6.61)	Li (6), anticonvulsants (7), typical (2) and atypical (12) antipsychotics, antidepressants (9), benzodiazepines (4)	SCID	3/19 eating disorders 5/19 substance abuse 11/19 anxiety disorders	Multi-episode unspecified
Jogia et al. (2008)	YMRS score BD:<7 HDRS score BD:<14	Unspecified	anticonvulsants (12)	SCID	No comorbid axis I and II disorders	Mean total episodes 10.1 (6.5)
Killgore et al.(2008)	YMRS score BD: 14.3 (8.9) HDRS score BD: 15.6 (9.9)	<1 year	Li or anticonvulsants (5), atypical antipsychotics (12), antidepressants (1), benzodiazepines (4)	SCID	Unspecified	First hospitalisation
Lawrence et al. (2004)	BDI score BD: 15.3 (9.2) MDD: 31.8 (11.8)	BD: 15.4 (13.4) MDD: 8 (5)	Li (3), antiepileptics (7), atypical antipsychotics (5), antidepressants (5)	Unspecified	No comorbid axis I and II disorders	Multi-episode unspecified
Lee et al. (2008)	HDRS score MDD: 22.2 (4)	MDD: 14.9 (8.8)	Antidepressants (10)	SCID	No comorbid axis I and II disorders	Mean depressive episodes 1.9 (0.8)
Lennox et al. (2004)	YMRS score BD: 27.7 (7.9) HDRS score BD: 0 (0)	Unspecified	Li (8), antiepileptics (7), typical antipsychotics (4), atypical antipsychotics (3)	Unspecified	Unspecified	Unspecified
Malhi et al. (2007)	YMRS score BD: <6 HDRS score BD:<6	BD: 12 (7.7)	Li (3), anticonvulsants (5)	SCID	No comorbid axis I and II disorders	Mean depressive episodes 10.4 (8.7) Mean manic episodes 4.7 (3.4)
Norbury et al. (2010)	BDI score MDD: 3.5 (3.7)	Unspecified	medication free	Unspecified	1/16 anxiety disorders 2/16 alcohol misuse	Patients with at least 2 previous episode of depression

(continued on next page)

Table 2 (*continued*)

Study	Psychopathology measures Mean, total score (standard deviation)	Duration of illness Mean, years (standard deviation)	Psychotropic medication (number of patients)	Diagnostic instrument	Comorbidity (number of patients with comorbidity/ total sample)	First or multiple episodes
Scheuerecker et al. (2010)	HDRS score MDD: 20.5 (4.7)	MDD: 52.3 (71.5)	Medication free	SCID	No comorbid axis I and II disorders	8 patients with first episodes and 5 with recurrent episodes Mean depressive episodes 1.4 (0.6)
Suslow et al. (2010)	HDRS score MDD: 24.8 ± 4.9	MDD: 6 (6.2)	Antidepressants (30)	SCID	13/30 anxiety disorders 3/30 dysthymia 1/30 pain disorder	Mean depressive episodes 2.7 (2)
Thomas et al. (2010)	MADRS score MDD: 2.3 (3.2)	Unspecified	Antidepressants (3)	SCID	No comorbid axis I and II disorders	Unspecified
Townsend et al. (2010)	HDRS score MDD: 20.1 (4.9)	MDD: 14.7 (13.3)	Medication free	SCID	No comorbid axis I and II disorders	Median depressive episodes 3
Van Wingen et al. (2010)	HDRS score MDD (depressed): 21.8 (4.2) MDD (recovered): 3.3 (2)	Unspecified	Medication free	SCID	No comorbid axis I and II disorders	18 with first MDE and 18 recovered from a first MDE

ASRM = Altman Self-Rating Mania Scale; BD = Bipolar Disorder; BDI = Beck Depression Inventory; HDRS = Hamilton Depression Rating Scale; MDD = Major Depressive Disorder; SCID = Structured Clinical Interview for DSM-IV; YMRS = Young Mania Rating Scale.

Table 3 Results from the global Activation Likelihood Estimation (ALE) analyses of facial affect processing in Bipolar Disorder and Major Depressive Disorder ($p<0.05$ False Discovery Rate corrected).

Brain region	Side	Centre of maximum ALE			Volume, mm^3	Maximum ALE value
		X	Y	Z		
Bipolar Disorder>healthy controls						
Parahippocampal gyrus	L	−18	−4	−16	1048	0.01
Parahippocampal gyrus		−18	−18	−8		
Putamen		−26	−6	−8		
Parahippocampal gyrus	R	24	−4	−14	392	0.01
Thalamus (Pulvinar)	L	−6	−26	4	368	0.01
Healthy controls>Bipolar Disorder						
Inferior frontal gyrus	L	−34	26	−8	480	0.01
	R	36	30	−8	872	0.01
Major Depressive Disorder>healthy controls						
Parahippocampal gyrus (amygdala)	R	28	0	−16	232	0.01
		30	−4	−20		
Healthy controls>Major Depressive Disorder						
Putamen	R	28	−1	0	400	0.01
Caudate	L	−36	−14	−10	256	0.01
Bipolar Disorder>Major Depressive Disorder						
Parahippocampal gyrus (amygdala)	L	−18	−4	−14	2984	0.01
		−18	−30	−8		0.008
		−24	6	−16		0.006
	R	22	−6	−12	376	0.008
Thalamus (Pulvinar)	L	−6	−26	6	584	0.01
Anterior cingulate gyrus (ventral)	R	8	24	24	376	0.007
	R	6	30	28		
Major Depressive Disorder>Bipolar Disorder						
Anterior cingulate gyrus (dorsal)	L	−20	−18	46	416	0.007

L=left; R=right; x=sagittal, y=coronal, z=axial coordinates according to Talairach and Tournoux.

computed. ALE scores from the convergent MA maps were then calculated on a voxel-by-voxel basis to test for convergent (random-effects) rather than study specific foci (fixed-effects). All ALE data processing was performed using the BrainMap Search and View software (http://brainmap.org).

First we performed two separate global meta-analyses (a) all studies comparing BD patients to controls, and (b) all studies comparing MDD patients to controls. Differences between diagnostic groups were tested by computing the voxel-wise difference between the ensuing ALE maps. Statistical inference was based on a threshold of $p<0.05$ with False Discovery Rate (FDR) correction and a minimum cluster size of 200 mm^3. At a second stage, we conducted a series of subsidiary meta-analyses within each diagnostic group depending on stimulus valence. Based on data availability, we focused on fearful and happy facial expressions as exemplars of negative and positive valence. For all analyses, all ALE maps were imported into Mango and overlaid on an anatomical template (http://ric.uthscsa.edu/mango/) for representation purposes. Coordinates of the maximum ALE and corresponding Brodmann areas are reported.

3. Results

We identified 37 studies that used facial affect paradigms in patients with BD or MDD of which twenty fulfilled all inclusion criteria, giving a total sample of 168 BD and 189 MDD patients and 344 healthy controls (HC) (Table 1). Excluded studies (a) grouped mood disorder patients together with other diagnostic groups (Lau et al., 2009), (b) grouped positive and negative facial stimuli together (Matthews et al., 2008; Yang et al., 2010; Anderson et al., 2011), (c) did not provide coordinates of the case–control comparison (Yurgelun-Todd et al., 2000; Gaffrey et al., 2010; Liu et al., 2010; Versace et al., 2010) or of the emotional versus neutral facial expressions contrast (Victor et al., 2010; Frodl et al., 2010), (d) did not include a control group (Keedwell et al., 2009), (e) implemented functional connectivity (Almeida et al., 2009) or pattern classification analyses (Fu et al., 2008), (f) used facial affect stimuli to examine other processes (e.g. interference) (Keedwell et al., 2005; Fales et al., 2008), and (g) examined the same patient group as other included studies (Haldane et al., 2008; Fu et al., 2004).

Demographic details for all participants and clinical information about BD and MDD patients in the studies included are shown in Table 2. As the definitions of participants' mental state differed in the primary studies we provided the mean psychopathology rating scales' scores (Table 2). With the exception of 5 studies which included medication free patients, patients received combinations of different psychotropics.

3.1. Global meta-analyses

3.1.1. Bipolar Disorder vs. controls

BD patients compared to controls showed (a) increased activation in the parahippocampal gyrus (extending to the amygdala) bilaterally, in the left putamen and left pulvinar (14 studies; 50 foci; 379 subjects) (Table 3), and (b) decreased activation bilaterally in the ventrolateral prefrontal cortex, within the inferior frontal gyrus (BA47) (Table 3) (Fig. 1).

3.1.2. Major Depressive Disorder vs. controls

MDD patients compared to controls showed (a) increased activation in the right parahippocampal gyrus (extending to the amygdala) (7 studies; 24 foci; 280 subjects) (Table 3), and (b) decreased activation in the right putamen, and left caudate (10 studies; 44 foci; 423 subjects) (Table 3) (Fig. 1).

3.1.3. Bipolar Disorder vs. Major Depressive Disorder

BD patients showed greater likelihood of activation than MDD patients in the parahippocampal gyrus (cluster included the amygdala), in the ventral anterior cingulate gyrus bilaterally and in the left pulvinar. Conversely, MDD patients had increased likelihood of activation than BD patients in dorsal anterior cingulate gyrus (Table 3).

3.2. Stimulus valence sub-analyses

3.2.1. Fear faces

3.2.1.1. Bipolar Disorder. In the fearful > non emotional stimuli contrast BD patients, compared to controls, showed (a) increased activation in the left parahippocampal gyrus (BA 28 and 35), left putamen and left pulvinar thalamus (7 studies; 30 foci; 166 subjects), and (b) decreased activation in the inferior frontal gyrus (BA47/45) bilaterally and in the left anterior cingulate gyrus (BA32) (7 studies; 23 foci; 170 subjects) (Table 4).

3.2.1.2. Major Depressive Disorder. In the same contrast (fearful > non emotional stimuli) (4 studies; 14 foci; 97 subjects) MDD patients showed decreased activation in sensorimotor cortices within the left precentral gyrus (BA6) (Table 4).

3.2.2. Happy faces

3.2.2.1. Bipolar Disorder. Compared to controls, in the happy > non emotional stimuli BD patients showed (a) increased activation in the caudate bilaterally and left parahippocampal gyrus (BA34) (4 studies; 16 foci; 132 subjects) and (b) decreased activation in the right anterior cingulate gyrus (BA32) (3 studies; 23 foci; 95 subjects) (Table 4).

3.2.2.2. Major Depressive Disorder. In the same contrast (happy > non emotional stimuli) (3 studies; 14 foci; 130 subjects) activation was decreased in the right pulvinar thalamus in MDD patients compared to controls (Table 4).

4. Discussion

The results of this meta-analysis provide evidence for common and distinct patterns of neural engagement in BD and MDD during facial affect processing. There are four key findings. First, both BD and MDD patients showed increased activation, relative to controls, in limbic regions, irrespective of stimulus valence. Second, BD was associated with reduced ventrolateral prefrontal cortical activation while MDD with decreased engagement of somatosensory cortices. Third, activation in the pulvinar thalamus and basal ganglia was increased in BD compared to controls and MDD patients. Fourth, these findings showed evidence of modulation by stimulus valence.

4.1. Increased limbic engagement: a common feature of BD and MDD

Our findings broadly confirm the prevailing view that mood disorders are associated with increased limbic activation during emotional processing (Savitz and Drevets, 2009). Our data question however the current "amygdalocentric" models for mood disorders. Clusters of abnormal medial temporal activation in both BD and MDD centred on the parahippocampal gyrus although they extended to include the amygdala. Chen et al. (2011) reported a similar pattern in a previous meta-analysis of fMRI studies in BD (Chen et al., 2011) and we now extend these observations to MDD. The parahippocampal gyrus and amygdala lie very close to each other and frequently co-activate during emotional processing (Fusar-Poli et al., 2009; Vytal and Hamann, 2010). Therefore the accuracy in locating the peak of activation within medial lobe structures could be influenced by smoothing and transforming data from individual subjects into a common stereotactic space. Other methodological considerations may relate to the type of paradigm or mood state. For example the amygdala become more engaged when facial expressions are processed outside the focus of attention (Hariri et al., 2003) and their activation may "normalise" with symptomatic remission, especially in MDD (Delaveau et al., 2011). Alternatively, it is possible that our results genuinely reflect greater parahippocampal involvement, relative to the amygdala, in mood disorders. The parahippocampal gyrus and the amygdala are thought to subserve partially segregated dimensions of emotional processing; amygdala engagement may signal salience or ambiguity (Gerber et al., 2008; Santos et al., 2011) while parahippocampal activation may reflect context appraisal (Gerdes et al., 2010). Whether these processes are differentially affected in mood disorders requires further investigation. In any case our results add to the emerging consensus that a more detailed evaluation of the role of limbic structures in mood disorders is warranted and this should crucially involve a revaluation of the central role currently ascribed to the amygdala. This is timely as within the field of affective neuroscience the role of the amygdala is undergoing major reappraisal with greater emphasis being placed on the contribution of other cortical and subcortical structures (Pessoa and Adolphs, 2010).

4.2. Distinct cortical involvement in BD and MDD

BD, but not MDD, was associated with reduced engagement in ventrolateral prefrontal regions within the inferior frontal gyrus. The ventrolateral prefrontal cortex is involved in inhibitory control across a number of paradigms including

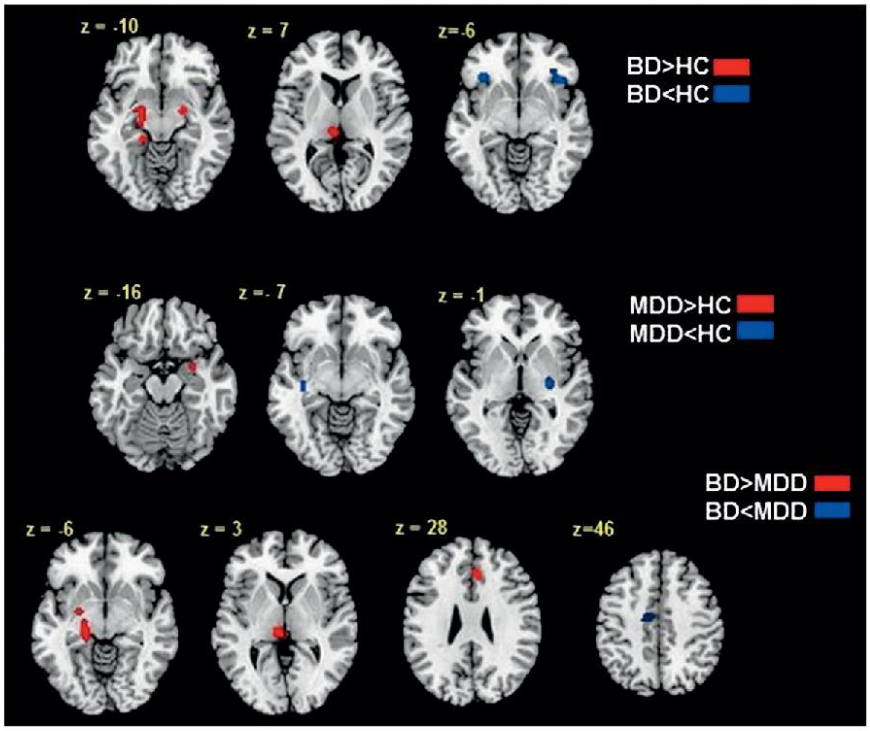

Figure 1 Activation Likelihood Estimation (ALE) maps representing regional activity consistently associated with Bipolar Disorder (BD) or Major Depressive Disorder (MDD). Clusters of relative overactivation or underactivation are shown in red and blue respectively; numbers represent axial (z) coordinates of each slice in Talairach space; $p<0.05$ False Discovery Rate corrected for multiple comparisons. Top row: statistical map of significant ALE clusters for the comparison of BD patients to healthy controls (HC). Middle row: statistical map of significant ALE clusters for the comparison of MDD patients to healthy controls (HC). Bottom row: statistical maps of significant ALE clusters associated with the contrast of BD and MDD.

emotional processing (Quirk and Beer, 2006). Dysfunction within this region is therefore thought to reflect reduced inhibitory capacity in BD (Cerullo et al., 2009; Chen et al., 2011). The degree of dysfunction in this region may be modulated by valence as it was most consistently observed when BD patients processed negative facial expressions. Ventrolateral PFC engagement regulates stimulus-driven action by modulating the influence of emotional stimuli on cognition with respect to contextually (or socially) appropriate behaviour (Quirk and Beer, 2006). In this respect ventrolateral PFC dysfunction may be relevant to stimulus-driven, socially inappropriate behaviour observed during mania.

Further diagnosis-related differences were observed in somatosensory regions where MDD patients showed decreased responsiveness compared to healthy individuals, particularly when viewing negative facial expressions. Somatosensory cortices contribute to the recognition of facial emotions (Adolphs, 2002) possibly through a process of invoking or "mirroring" internal representations of the pertinent emotional experience (Adolphs et al., 2000). Since MDD patients experience negative emotions frequently as part of the clinical syndrome of depression this finding could be suggestive of adaptive down-regulation of processing of negative stimuli. Similar observations of reduced emotional reactivity in MDD have been made previously in a variety of experimental settings and are thought to reflect emotion-context insensitivity (Rottenberg et al., 2005).

4.3. Common and distinct thalamic engagement in BD and MDD

Our results suggest that thalamic involvement in mood disorders is complex. Increased thalamic engagement was uniquely associated with BD. However, thalamic activation may be influenced by stimulus valence. Increased pulvinar activation was observed in BD during the processing of negative facial expressions while decreased activation in the same nucleus was noted in MDD during the processing of happy faces. The pulvinar is considered a "higher order"

Table 4 Results from the valence Activation Likelihood Estimation (ALE) sub-analyses of facial affect processing in Bipolar Disorder and Major Depressive Disorder ($p<0.05$ False Discovery Rate corrected).

Brain region	Side	Centre of maximum ALE			Volume, mm^3	Maximum ALE value
		X	Y	Z		
Fear						
Bipolar Disorder>healthy controls						
Parahippocampal Gyrus	L	−18	−12	−12	584	0.01
Putamen	L	−26	−6	−8		0.008
Thalamus (Pulvinar)	L	−6	−26	4	376	0.01
Healthy controls>bipolar disorder						
Inferior frontal Gyrus (BA47)	L	−34	24	−8	480	0.01
	R	44	22	−2	1080	0.01
Inferior frontal gyrus (BA45)	R	52	12	28	408	0.01
Anterior cingulate (BA32)	L	−8	34	12	424	0.01
Major Depressive Disorder>healthy controls						
No suprathreshold clusters						
Healthy controls>Major Depressive Disorder						
Precentral gyrus	L	−58	0	12	96	0.009
Happy						
Bipolar Disorder>healthy controls						
Caudate	L	−18	20	14	80	0.009
	R	14	10	16	96	0.009
		18	24	−6	80	0.009
Parahippocampal gyrus	L	−26	6	−16	80	0.008
Healthy controls>Bipolar Disorder						
Anterior cingulate gyrus	R	20	14	34	80	0.009
Major Depressive Disorder>healthy controls						
No supratheshold clusters						
Healthy controls>Major Depressive Disorder						
Thalamus	R	6	−28	4	368	0.01

L=left; R=right; x=sagittal, y=coronal, z=axial coordinates according to Talairach and Tournoux.

nucleus because of its widespread bidirectional cortical connections (Pessoa and Adolphs, 2010). The pulvinar is directly involved in visual perception (Pessoa and Adolphs, 2010), particularly in directing and maintaining attention towards salient stimuli (Desimone et al., 1990). Our results suggest that in BD the pulvinar overactivation may act to amplify neural engagement for emotionally salient, particularly negative stimuli. The reverse appears to be the case in MDD when processing happy faces; this is in line with current views that MDD may be characterised by reduced reactivity to positive stimuli (Rottenberg et al., 2005).

4.4. Distinct basal ganglia involvement in BD and MDD

Diagnosis related changes were also noted in the basal ganglia where increased engagement was observed in BD compared to controls while the reverse was the case for MDD. Specifically, BD patients expressed increased activation in the putamen and in the caudate in response to negative and positive facial expressions respectively. The putamen is mainly involved in sensorimotor processing and is thought to contribute to the motor production of facial expressions during negative affect recognition (Adolphs, 2002). Increased putamen activation in BD patients may reflect either greater facial mimicry or greater amplification of sensorimotor processing of negative facial affect. The latter interpretation is supported by the increased engagement of the pulvinar.

During happy facial affect processing BD patients, compared to controls, expressed increased activation in the caudate nucleus. This is in line with findings implicating the caudate in processing rewarding stimuli (Schultz et al., 1997; O'Doherty et al., 2003) including happy facial expressions (Phan et al., 2002). As activation in reward-circuitry structures correlates positively with valence (Gerdes et al., 2010) our results indicate that happy facial stimuli may have greater reward value for BD patients. This observation may relate to the inappropriate and generalised activation of reward-related structures in mania (Abler et al., 2008).

5. Methodological considerations

Activation Likelihood Estimation represents a powerful approach for the meta-analytic treatment of neuroimaging data. Still, a number of factors should be considered in the interpretation of the current set of findings. First, our initial review revealed great variability in the emotional processing paradigms used which coupled with small sample sizes impacts on the ability to draw statistically robust conclusions

from this literature. To minimise variability due to study design we focused exclusively on studies using comparable versions of facial affect processing tasks. Second, we included results reported as significant in the original studies since ALE analyses do not allow weighting based on the threshold of significance employed in each individual study. Third, there was significant variability in the level of patients' symptomatology at the time of testing and in the definition of "remitted" or "euthymic" states (Table 2). Separate analyses of the available studies according to mood states would not have been statistically feasible. Therefore, the changes in regional brain activity identified here cannot be clearly categorised as trait or state. Fourth, as shown in Table 2, with the exception of 5 studies, patients were medicated and were prescribed combinations of psychotropics. Given the inter-study variability in medication regimes a systematic bias influencing our results is improbable. Additionally psychotropic medication predominantly acts to reduce case–control differences in neural activity in mood disorders (Phillips et al., 2008; Delaveau et al., 2011). Fifth, sex differences have been found during facial affect processing (Fusar-Poli et al., 2009). Although it was not possible to examine this directly, the original studies included samples that were generally balanced and matched for sex (Table 1), thus minimising the likelihood of a systematic sex-related bias confounding the effect of diagnosis. Sixth, since most original studies examined multi-episode BD or MDD patients (Table 2), further investigation is required to clarify the relevance of our results to the initial stages of mood disorders. Seven, current meta-analytic algorithms cannot adequately address the effect of demographic, behavioural or clinical variables on the distribution of the reported brain activation patterns. Such questions would be ideally investigated in large neuroimaging data sets where the quantitative impact of these variables could be directly assessed. Finally, this meta-analysis provides an estimate of the probability that activity in particular brain regions may differ between groups (e.g. BD vs. controls). It is not an estimate of the mean difference in regional signal change and therefore traditional measures of heterogeneity and publication bias that are based on effect size are not applicable. Although we cannot exclude a publication bias against negative studies, this would have had no effect on the current results.

6. Conclusion and future directions

In conclusion, we provided evidence for common and distinct neural correlates in BD and MDD in response to emotional faces. Our results point to three new avenues of enquiry in mood disorders. Firstly, they suggest the need for more detailed examination of the relative contribution of medial temporal regions and particularly the interaction between amygdala and parahippocampus. Second, they underscore the contribution of cortical, thalamic and basal ganglia regions to the pathophysiology of mood disorders and suggest that examination of these cortico-thalamic-basal ganglia circuits may shed light to mechanisms differentiating BD from MDD. Third, they point to stimulus valence as an important modulator of activity within the neural networks underlying emotional processing in mood disorders.

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Contributors

All authors contributed to and have approved the final manuscript.

Conflict of interest

All authors declare no conflict of interest.

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Appendix 3: Original paper on: **"Evidence of diagnostic specificity in the neural correlates of facial affect processing in bipolar disorder and schizophrenia: a meta-analysis of functional imaging studies." Psychological Medicine (2013), 43, 553–569.**

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ORIGINAL ARTICLE

Evidence of diagnostic specificity in the neural correlates of facial affect processing in bipolar disorder and schizophrenia: a meta-analysis of functional imaging studies

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Background. Schizophrenia (SZ) and bipolar disorder (BD) may overlap in etiology and phenomenology but differ with regard to emotional processing. We used facial affect as a probe for emotional processing to determine whether there are diagnosis-related differences between SZ and BD in the function of the underlying neural circuitry.

Method. Functional magnetic resonance imaging (fMRI) studies published up to 30 April 2012 investigating facial affect processing in patients with SZ or BD were identified through computerized and manual literature searches. Activation foci from 29 studies encompassing 483 healthy individuals, 268 patients with SZ and 267 patients with BD were subjected to voxel-based quantitative meta-analysis using activation likelihood estimation (ALE).

Results. Compared to healthy individuals, when emotional facial stimuli were contrasted to neutral stimuli, patients with BD showed overactivation within the parahippocampus/amygdala and thalamus and reduced engagement within the ventrolateral prefrontal cortex (PFC) whereas patients with SZ showed underactivation throughout the entire facial affect processing network and increased activation in visual processing regions within the cuneus. Patients with BD showed greater thalamic engagement compared to patients with SZ; in the reverse comparison, patients with SZ showed greater engagement in posterior associative visual cortices.

Conclusions. During facial affect processing, patients with BD show overactivation in subcortical regions and underactivation in prefrontal regions of the facial affect processing network, consistent with the notion of reduced emotional regulation. By contrast, overactivation within visual processing regions coupled with reduced engagement of facial affect processing regions points to abnormal visual integration as the core underlying deficit in SZ.

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Key words: Amygdala, cuneus, facial affect, psychosis, visual processing.

Introduction

Bipolar disorder (BD) and schizophrenia (SZ) are currently classified as separate diagnostic entities (APA, 1994; WHO, 2004). Although this distinction may be justifiable and clinically useful (Lawrie *et al*. 2010), it has been questioned on the basis of significant overlap between the two disorders in terms of phenomenology (Fischer & Carpenter, 2009), genetic risk factors (International Schizophrenia Consortium, 2009; Lichtenstein *et al*. 2009) and brain morphological changes (Ellison-Wright & Bullmore, 2010; Yu *et al*. 2010).

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In this study we focus specifically on facial affect processing and examine whether diagnosis-related differences in the engagement of the corresponding neural network support the distinction between SZ and BD. The reasons for this are threefold. First, differences in emotional processing have been highlighted as the most distinctive features differentiating SZ from BD. Both early descriptions and later investigations of the two syndromes (Bleuler, 1950; Kraepelin, 1971; Carpenter *et al*. 1973) emphasize avolition and restricted affect in SZ and excess emotional reactivity in BD. Second, facial affect is the most common probe of the neural circuitry involved in emotional processing because of its biological and behavioral salience (LeDoux, 1995; Adolphs, 2002). Facial affect processing engages numerous neural systems with key nodes spatially distributed within the primary and extrastriate visual cortices (particularly within the fusiform gyrus) in the occipital and ventral

temporal lobes, the superior temporal sulcus, the medial and ventrolateral prefrontal cortex (PFC), limbic (amygdala and insula, among others) and thalamic areas (Adolphs *et al.* 1996; Haxby *et al.* 2002; Phan *et al.* 2002; Murphy *et al.* 2003; Fairhall & Ishai, 2007; Fusar-Poli *et al.* 2009; Vytal & Hammann, 2010; Dima *et al.* 2011). Most of these regions are also implicated in current models of BD and SZ that respectively highlight abnormal interaction within prefrontal–subcortical–limbic (Strakowski *et al.* 2005) and prefrontal–temporal–subcortical–limbic networks (Gur *et al.* 2007*a*). Third, available findings in BD and SZ are suggestive of diagnosis-specific differences, particularly within limbic and posterior parieto-occipital regions. For example, viewing emotional faces compared to neutral faces is associated with increased engagement of the parahippocampus/amygdala in BD (Altshuler *et al.* 2005; Strakowski *et al.* 2005; Chen *et al.* 2006; Foland *et al.* 2008; Delvecchio *et al.* 2012) but not in SZ (Aleman & Kahn, 2005; Holt *et al.* 2006; Surguladze *et al.* 2006; Hall *et al.* 2008; Seiferth *et al.* 2009; Li *et al.* 2010; Anticevic *et al.* 2012). Additionally, recruitment in medial occipital and parietal regions involved in visual processing has been reported to be enhanced in SZ (Farkas *et al.* 1984; Taylor *et al.* 2012) and reduced in BD (Pavuluri & Passarotti, 2008). However, inferring potential differences or similarities between the two disorders from existing studies is difficult because direct comparisons between the diagnostic groups are limited. Therefore, meta-analytic techniques are currently the best option to investigate brain regions differentially engaged in SZ and BD, and thus address questions of diagnostic specificity.

We conducted a quantitative voxel-based meta-analysis of available functional magnetic resonance imaging (fMRI) data to test for diagnosis-specific dysfunction in the neural correlates of facial affect processing in SZ and BD. Our key prediction was that functional disruption in limbic and associated areas would differ between the two disorders. We also hypothesized that SZ, but not BD, would be associated with increased engagement of posterior midline cortical regions involved in higher-order visual processing.

Method

Data sources and inclusion criteria

Studies investigating facial affect processing in patients with SZ or BD were identified through computerized literature searches using Medline and PubMed supplemented by inspection of reference lists. We reviewed all papers published in the English language up to 30 April 2012. The search keywords used were 'bipolar disorder', 'schizophrenia', 'facial affect', 'facial emotion', 'emotional processing', 'facial affect labeling', 'facial affect matching' 'mania' and 'fMRI' and their combinations and differing terminations, in addition to terms specifying individual facial affects (e.g. fear, happiness). Studies were included if they (*a*) reported comparisons between SZ or BD patients and healthy individuals, (*b*) used fMRI in conjunction with subtraction methodology to identify foci of task-related neural changes contrasting an active (emotional faces) and a control condition (neutral faces or shapes or fixation cross), and (*c*) reported their results in standard stereotactic coordinates [either Talairach or Montreal Neurological Institute (MNI) space]. Studies were excluded if they used facial stimuli to investigate processes not directly involved in emotional processing (e.g. working memory, attention) or if they focused on pediatric or geriatric patients.

Activation likelihood estimation (ALE) procedure

Meta-analyses were performed using the ALE software implemented in GingerALE 2.0.4 (http://brainmap.org). This version uses a random effect model and weighting for sample size of the original studies (Laird *et al.* 2005). The contrasts included are shown in Table 1. We accepted results as significant based on the threshold used in the original studies. When both were available, coordinates used derived from whole-brain and not region of interest (ROI) analyses. Coordinates of the foci of activation reported in the original studies were transformed into Talairach space using the Lancaster transform (icbm2tal) in GingerALE. Activation coordinates derived from each study were weighted to yield estimates of activation likelihood at each voxel that were then combined to compute a modeled activation (MA) map. ALE scores from the convergent MA maps were then calculated on a voxel-by-voxel basis to test for convergent (random effects) rather than study-specific foci (fixed effects). All ALE data processing was performed using the BrainMap Search and View software (http://brainmap.org). The threshold of statistical significance was set at $p<0.05$, with false discovery rate (FDR) correction for multiple comparisons and a minimum cluster size of 200 mm^3. Each ALE map was imported into Mango (http://ric.uthscsa.edu/mango) and overlaid on an anatomical template (www.brainmap.org/ale/colin1.1.nii) for representation purposes. For each suprathreshold cluster, corresponding Brodmann areas (BAs) were identified using the Talairach and Tournoux stereotactic anatomic brain atlas (Talairach & Tournoux, 1988). Finally, to compare diagnostic

Table 1. *Details of studies included in the meta-analysis (in alphabetical order)*

Reference	Sample (M/W)	Task	Design	Contrast
Schizophrenia (SZ)				
Das *et al.* (2007)	14 SZ (14/0) 14 HS (14/0)	Implicit facial affect viewing and explicit facial affect labeling task	Block ROI analysis	Fearful > Neutral
Dowd & Barch (2010)	32 SZ (20/12) 40 HS (26/14)	Explicit valence rating task	Event related Whole-brain and ROI analyses	Fearful > Neutral
Fakra *et al.* (2008)	14 SZ (9/5) 14 HS (9/5)	Explicit facial affect matching task	Block Whole-brain analysis	Fearful and angry > Shapes
Gur *et al.* (2002)	14 SZ (10/4) 14 HS (10/4)	Implicit emotional valence discrimination	Block Whole-brain analysis	Fearful, sad, angry, happy, disgust > Fixation cross
Gur *et al.* (2007*b*)	16 SZ (12/4) 17 HS (12/5)	Explicit facial affect labeling task	Event related Whole-brain analysis	Fearful > Neutral Angry > Neutral
Habel *et al.* (2010)	17 SZ 17 HS Sex unspecified	Explicit facial affect labeling task	Event related Whole-brain analysis	Fearful > Baseline Angry > Baseline Happy > Baseline Sad > Baseline
Hall *et al.* (2008)	19 SZ (12/7) 24 HS (16/8)	Implicit facial affect recognition	Block ROI analysis	Fearful > Neutral
Hempel *et al.* (2003)	9 SZ (4/5) 10 HS (6/4)	Explicit facial affect labeling task	Block Whole-brain analysis	Fearful, sad, angry, happy, disgust and surprise > Baseline
Holt *et al.* (2006)	15 SZ (15/0) 16 HS (16/0)	Implicit facial affect recognition	Block ROI analysis	Fearful > Neutral Happy > Neutral
Kosaka *et al.* (2002)	12 SZ (6/6) 12 HS (6/6)	Emotional intensity judgment task	Block ROI analysis	Happy > Neutral
Lepage *et al.* (2011)	26 SZ (11/15) 26 HS (14/12)	Implicit facial emotion perception	Event related Whole-brain analysis	Sad and happy > Neutral
Li *et al.* (2012)	12 SZ (6/6) 12 HS (6/6)	Explicit facial emotional valence discrimination	Event related Whole-brain analysis	Happy > Neutral Fearful > Neutral
Michalopoulou *et al.* (2008)	11 SZ (9/2) 9 HS (5/4)	Implicit facial affect recognition	Event related Whole-brain analysis	Fearful > Neutral
Rauch *et al.* (2010)	12 SZ (7/5) 12 HS (9/3)	Implicit facial affect recognition	Block ROI analysis	Happy > Neutral Sad > Neutral
Reske *et al.* (2009)	18 SZ (10/8) 18 HS (10/8)	Explicit facial affect labeling task	Event related Whole-brain analysis	Sad > Neutral
Williams *et al.* (2007)	13 SZ (8/5) paranoid 14 SZ (9/5) non-paranoid 13 HS (8/5)	Implicit facial affect recognition	Block Whole-brain analysis	Fearful > Neutral
Bipolar disorder (BD)				
Almeida *et al.* (2010)	15 BD remitted (5/10) 15 BD depressed (1/14) 15 HS (3/12)	Explicit facial affect labeling task	Event related Whole-brain and ROI analyses	Sad > Neutral
Altshuler *et al.* (2008)	11 BD (5/6) 17 HS (9/8)	Explicit facial affect matching task	Block Whole-brain analysis	Fearful and angry > Shapes
Blumberg *et al.* (2005)	17 BD (10/7) 17 HS (7/10)	Implicit facial affect recognition	Block ROI analysis	Happy > Fixation
Chen *et al.* (2006)	8 BD depressed (5/3) 8 BD manic (8/0) 8 HS (2/6)	Explicit facial affect labeling task and implicit facial affect recognition	Event related Whole-brain analysis	Fearful > Neutral (explicit) Happy > Neutral (explicit)

Table 1 (*cont.*)

Reference	Sample (M/W)	Task	Design	Contrast
Foland *et al.* (2008)	9 BD (3/6) 9 HS (3/6)	Explicit facial affect matching task	Block Whole-brain analysis	Fearful and anger > Shapes
Foland-Ross *et al.* (2012)	24 BD (15/9) 26 HS (15/11)	Explicit facial affect matching task	Block Whole-brain analysis	Label emotions > Shapes
Hassel *et al.* (2008)	19 BD (10/9) 24 HS (11/13)	Implicit facial affect recognition	Event related Whole-brain and ROI analyses	Happy > Neutral
Hulvershorn *et al.* (2012)	30 BD depressed (18/12) 30 BD manic (19/11) 15 BD euthymic (15/0) 30 HS (19/11)	Explicit facial affect matching task	Block Whole-brain analysis	Emotional faces > Shapes
Jogia *et al.* (2008)[a]	12 BD (5/7) 12 HS (5/7)	Explicit facial affect labeling task	Event related Whole-brain analysis	Sad > Neutral Angry > neutral
Killgore *et al.* (2008)	14 BD (11/3) 13 HS (12/1)	Explicit face perception task	Block Whole-brain and ROI analyses	Fearful > fixation
Lawrence *et al.* (2004)	20 BD 11 HS 60 % M	Implicit facial affect recognition task	Event related ROI analysis	Fearful > Neutral Happy > Neutral Sad > Neutral
Lennox *et al.* (2004)	10 BD (8/2) 12 HS (6/6)	Explicit facial affect rating task	Event related Whole-brain and ROI analyses	Sad > Neutral
Malhi *et al.* (2007)	10 BD (0/10) 10 HS (0/10)	Explicit facial affect labeling task	Event related Whole-brain and ROI analyses	Fearful > Neutral

HS, Healthy subjects; M, men; W, women; ROI, region of interest
[a] Only baseline data used.

groups, the ALE maps for SZ and BD were contrasted directly using the subtraction meta-analysis procedure implemented in GingerALE. We report only results where there was convergence of activation between two or more studies.

Moderator variables

Demographic information about age and sex and clinical information about level of psychopathology and medication type and dosage were extracted from each primary study. Mean antipsychotic dose was converted to chlorpromazine equivalents (Bezchlibnyk-Butler & Jeffries, 2010). The effect of other medications (e.g. lithium) was not examined because dosage was not mentioned in a sufficient number of studies. We extracted information about participants' scores on the Hamilton Depression Rating Scale (HAMD; Hamilton, 1960), the Young Mania Rating Scale (YMRS; Young *et al.* 1978) and the positive and negative subscales of the Positive and Negative Syndrome Scale (PANSS; Kay *et al.* 1987). We tested for potential group differences in these moderator variables and examined their influence on the ALE results using meta-regression analyses. The impact of symptom severity was examined in each diagnostic group separately. Based on procedures implemented in previous studies (Van Snellenberg *et al.* 2006; Anticevic *et al.* 2012), we rescaled each psychopathology scale from 0 to 1, where 0 and 1 indicated respectively the minimum and maximum possible scores on each scale.

Results

Included studies

The study selection flowchart is shown in the PRISMA diagram provided in Fig. 1 and Table 1 shows the details of the studies. The total sample comprised 268 patients with SZ, 267 BD patients and 483 healthy individuals. Demographic details for all participants and clinical information of SZ and BD patients are shown in Table 2. There was a significant difference between the two patient groups in age (SZ: mean = 31.69 years, S.D. = 6.1 years; BD: mean = 35.9 years, S.D. = 4.1; $t = -2.33$ $p = 0.026$) but not sex

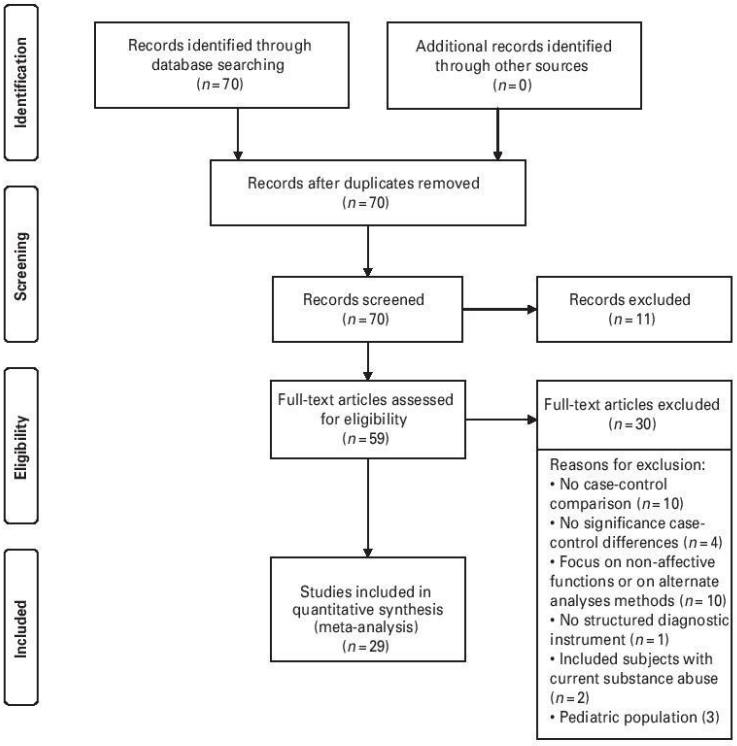

Fig. 1. Flowchart of study selection.

(SZ: 64.3% men, 35.7% women: BD: 56.2% men, 43.8% women, $\chi^2=3.55$, $p=0.06$).

Comparison of patients with SZ to healthy individuals

Compared to healthy individuals, patients with SZ showed an increased likelihood of activation in the right cuneus and a decreased likelihood of activation in frontal regions [left precentral gyrus (BA 6) and left medial frontal gyrus (BA 9)], in limbic and paralimbic regions [right amygdala, right insula and left parahippocampal gyrus (BA 28) and anterior cingulate cortex (ACC; BA 32)], in occipital and occipitotemporal regions [right inferior occipital gyrus (BA 17) and right fusiform gyrus (BA 37)], in the basal ganglia (right caudate nucleus) and in the right medial dorsal thalamus (19 studies; 103 foci and 645 subjects). Details are shown in Fig. 2 and Table 3.

No effect of gender was observed but age was positively correlated with the likelihood of activation in the left parahippocampal gyrus (BA 28) ($x=-20$, $y=-26$, $z=-8$; $r^2=0.66$, $p=0.001$, voxels=227). A higher PANSS positive symptoms subscale score correlated with a reduced likelihood of activation in the hippocampus/parahippocampal gyrus ($x=38$, $y=-18$, $z=-14$; $r^2=0.56$; $p=0.001$, voxels=619). A higher PANSS negative symptoms subscale score correlated with a reduced likelihood of activation in the precentral gyrus (BA 6) ($x=-38$, $y=2$, $z=40$; $r^2=0.56$, $p=0.001$, voxels=33).

Comparison of patients with BD to healthy individuals

Compared to healthy individuals, BD patients showed increased activation in the parahippocampal gyrus extending to the amygdala bilaterally and left pulvinar thalamus (15 studies; 51 foci and 473 subjects). Additionally, BD patients showed decreased activation in the inferior frontal gyrus bilaterally (BA 47, BA 44), the left ACC (BA 32) and the right caudate (13 studies; 47 foci and 366 subjects). Details are shown in Fig. 2 and Table 3. Age and sex did not

Table 2. *Description of participants included in the meta-analysis (in alphabetical order)*

Reference	Age (years) Mean (S.D.)	Co-morbidity	Symptom scores Mean (S.D.)	Symptomatic state[c]	Number of episodes Duration of illness (DI) Mean (S.D.)	Psychotropic medication (no. of patients)
***Schizophrenia* (*SZ*)**						
Das *et al.* (2007)	SZ: 20.4 (3.3) HS: 23.1 (5.9)	No substance abuse	PANSS Positive: 16.07 (7.24) PANSS Negative: 21.14 (7.9)	Remitted	First-episode psychosis DI: 1.21 (1.2) years	SGA (9)
Dowd & Barch (2010)	SZ: 36.25 (10.85) HS: 36.8 (8.99)	No substance abuse No co-morbid Axis I disorders	SAPS: 1.83 (.93) SANS: 1.81 (1.37)	Remitted	Unspecified DI: 17.73 (11.25) years	SGA and FGA (unspecified)
Fakra *et al.* (2008)	SZ: 34.64 (5.96) HS: 37.29 (8.87)	No substance abuse	PANNS Positive: 24.71 PANSS Negative: 13.14 (5.46)	Remitted	Unspecified	Antipsychotics (unspecified)
Gur *et al.* (2002)	SZ: 28.8 (8.9) HS: 27.4 (7.3)	No co-morbid Axis I and II disorders	SAPS: 0.5 (5) HAMD: 6.0 (4.7)	Remitted	Unspecified	FGA (1), SGA (11)
Gur *et al.* (2007*b*)	SZ: 30.1 (6.5) HS: 25 (3.9)	No co-morbid Axis I and II disorders	SAPS: 1.4 (0.6) SANS: 1.3 (0.9)	Remitted	Unspecified DI: 9.6 (7.1) years	FGA (2), SGA (11), Combination of FGA and SGA (2)
Habel *et al.* (2010)	SZ: 34.4 (8.8) HS: 34.2 (7.7)	No co-morbid Axis I and II disorders	PANSS Positive: 18.0 (7.3) PANSS Negative: 19.9 (8.8) PANSS General:76.5 (26.3)	Remitted	Unspecified	FGA (2), SGA (12), combination of FGA and SGA (2), unmedicated (1)
Hall *et al.* (2008)	SZ: 37.7 (8.4) HS: 35.1 (9.7)	No substance abuse No co-morbid Axis I and II disorders	PANSS Positive: 12.3 (4.5)	Remitted	Unspecified	FGA (3) and SGA (16)
Hempel *et al.* (2003)	SZ: 28 HS: 26	No substance abuse	PANSS Positive: 13 PANSS Negative: 13 PANSS General: 18	Remitted	Unspecified DI: 13 months	SGA (9)
Holt *et al.* (2006)	SZ: 47.7 (7.1) HS: 48.2 (9.6)	No substance abuse	PANSS Total: 59.8 (10.3)	Remitted	Unspecified DI: 21.6 (9.6) years	Unspecified
Kosaka *et al.* (2002)	SZ: 26 (4.5) HS: 24.4 (2.4)	Unspecified	PANSS Positive: 11.3 (4.6) PANSS Negative: 16.3 (4.5) PANSS General: 28.8 (7.3)	Remitted	Unspecified; DI: 3.8 (3.5) years	Antipsychotics (unspecified)
Lepage *et al.* (2011)	SZ: 31.8 (7.7) HC: 28.3 (5.6)	No co-morbid Axis I Disorders	SAPS: 9.6(10.2) SANS: 15.6(9.1)	Remitted	Unspecified; DI: 8.5 (6.5) years	SGA (18), combination of SGA and FGA (6), anticholinergic medication (1), combination of SGA/FGA and antidepressants (7)
Li *et al.* (2012)	SZ: 29.8 (9.24) HS: 29.25 (7.24)	No co-morbid Axis I and II disorders	PANSS Positive 16.08 (5.66) PANSS Negative: 13.41 (8.89) PANSS Total: 28.42 (13.82)	Remitted	Unspecified DI: 5.42 (3.75) years	Antipsychotic unspecified)
Michalopoulou *et al.* (2008)	SZ: 35 (9) HS: 32 (6)	No substance abuse	PANSS Positive: 16 (6.72) PANSS Negative: 13.91 (5.54) PANSS Total: 58.91 (17.72)	Remitted	Unspecified; DI: 12 (9) years	FGA (8), SGA (3)
Rauch *et al.* (2010)	SZ: 27.7 (7.5) HS: 26.9 (6.1)	No substance abuse No co-morbid Axis I disorders	PANSS Positive: 14.4 (3.1) PANSS Negative: 18.9 (5.2) PANSS General: 34.9 (5.9)	Remitted	Unspecified	SGA (9), combination of FGA and SGA (3)
Reske *et al.* (2009)	SZ: 31.94 (6.41) HS: 31.94 (6.03)	No co-morbid Axis I disorders	PANSS Positive: 8 (1.14) PANSS Negative: 13.61 (4.47) PANSS Total: 23.11 (3.94)	Remitted	First-episode psychosis Unspecified	FGA (9), SGA (9)
Williams *et al.* (2007)	SZ: 26.9 (9.1) paranoid SZ: 27.8 (10.4) non-paranoid HS: 25.1 (8.1)	No substance abuse	PANSS Delusions: 2.1 (1.1); Excitement1.6 (0.8)	Remitted	Unspecified DI: 5.6 (4.6) years	SGA
***Bipolar disorder* (*BD*)**						
Almeida *et al.* (2010)[a]	BD remitted: 33.28 (7.83) BD depressed: 36.56 (11.88) HS: 32.69 (8.00)	9/30 history of substance abuse	HAMD BD depressed: 21.53 (6.40) BD remitted: 1.47 (1.13)	Depressed and Remitted	All patients had experienced at least two episodes of illness in 4 years DI: BD depressed: 14.23 (9.82) years BD remitted: 14.67 (5.48) years	Unspecified
Altshuler *et al.* (2008)	BD: 32 (7.3) HS: 29.5 (6.6)	No substance abuse No co-morbid Axis I disorder	HAMD BD: 20.8 (3.3) YMRS BD: 2.9 (1.9)	Depressed	Unspecified	Unmedicated (2), anticonvulsants (7), Li (1), SGA (2), antidepressants (3)
Blumberg *et al.* (2005)	BD: 40.0 (12.3) HS: 33.2 (10.8)	9/12 history of alcohol dependence	HAMD CARSM Unspecified scores	Unspecified	Unspecified	Unmedicated (5), anticonvulsants (8), Li (4), antidepressants (3), SGA (1)

Table 2 (*cont.*)

Reference	Age (years) Mean (S.D.)	Co-morbidity	Symptom scores Mean (S.D.)	Symptomatic state[c]	Number of episodes Duration of illness (DI) Mean (S.D.)	Psychotropic medication (no. of patients)
Chen *et al.* (2006)	BD depressed: 41.88 (12.09) BD manic: 39 (13.44) HS: 38.75 (12.5)	No co-morbid Axis I and II disorders	YMRS BD manic: 24.13 (8.27) BD depressed: 0.43 (0.53) HAMD BD (manic): 2 (2.98) BD (depressed): 18.38 (6.44)	Manic	All patients had at least two previous episodes of mania and depression Unspecified	Li (11), anticonvulsants (9), antidepressants (2), FGA (2), SGA (3)
Foland *et al.* (2008)	BD: 34.6 (8.0) HS: 30.4 (7.6)	No substance abuse No co-morbid Axis I disorders	YMRS BD: 15.1 (3.7) HAMD BD: 9.1 (5.3)	Manic	Mean manic episodes: 4.2 (2) DI: 14.8 (5.1) years	Li (20), anticonvulsants (6), SGA (1)
Foland-Ross *et al.* (2012)	BD: 38.8 (12.8) HS: 37.9 (13.4)	No co-morbid Axis I disorders	YMRS: 1.5 (1.9) HAMD: 4.6 (2.1)	Remitted	Mean manic episodes: 6.3 (8.7) Mean depressive episodes: 9.6 (10.7); DI: 9.4 (11.0)	Anticonvulsants (10),SGA (11), antidepressants (6)
Hassel *et al.* (2008)	BD: 32.47 HS: 27.78	3/19 eating disorders 5/19 substance abuse 11/19 anxiety	YMRS BD: < 10 HAMD scores BD: < 7	Remitted	Multi-episodes unspecified DI: 10.6 (6.61) years	Li (6), anticonvulsants (7), FGA (2), SGA (12), antidepressants (9), benzodiazepines (4)
Hulvershorn *et al.* (2012)	30 BD depressed: 35 (11) 30 BD manic: 34 (11) 15 BD euthymic: 31 (11) 30 HS: 32 (10)	No substance abuse No co-morbid Axis I disorders	YMRS: BD depressed: 3 (3) BD manic: 16 (3) BD euthymic: 2 (3) HAMD: BD depressed: 20 (4) BD manic: 6 (3) BD euthymic: 7 (4)	Manic, depressive, remitted	Mean manic episodes BD depressed: 15 BD manic: 15 BD euthymic: 13 Mean depressive episodes BD depressed: 24 BD manic: 75 BD euthymic: 28; Unspecified	Unspecified
Jogia *et al.* (2008)[b]	BD: 42.1 (11.8) HS: 41.8 (10.9)	No substance abuse No co-morbid Axis I and II disorders	YMRS BD: < 7 HAMD BD: < 14	Remitted	Mean total episodes: 10.1 (6.5) Unspecified	Anticonvulsants (12)

Killgore *et al.* (2008)	BD: 28.1 (11.2) HS: 25.5 (4.7)	Unspecified	YMRS BD: 14.3 (8.9) HAMD BD: 15.6 (9.9)	Depressed	First hospitalization DI: < 1 year	Li or anticonvulsants (5) SGA (12), benzodiazepines (4), antidepressants (1)
Lawrence *et al.* (2004)[a]	Overall mean 41 (11) 60% Male	No substance abuse No co-morbid Axis I and II disorders	BDI BD: 15.3 (9.2)	Depressed	Multi-episodes Unspecified DI: BD: 15.4 (13.4) years unspecified	Li (3), anticonvulsants (7), SGA (5), antidepressants (5)
Lennox *et al.* (2004)	BD: 37.3 (12.8) HS: 32.6 (10.7)	Unspecified	YMRS BD: 27.7 (7.9) HDRS BD: 0 (0)	Depressed		Li (8), anticonvulsants (7), FGA (4), SGA (3)
Malhi *et al.* (2007)	BD: 33.5 (8.7) HS: 32.4 (6.4)	No substance abuse No co-morbid Axis I and II disorders	YMRS BD: < 6 HAMD BD: < 6	Remitted	Mean depressive episodes: 10.4 (8.7) Mean manic episodes: 4.7 (3.4) DI: 12.0 (7.7) years	Li (3), anticonvulsants(5)

BD, Bipolar disorder; SZ, schizophrenia; HS, healthy subjects; Li, lithium; BDI, Beck Depression Inventory; CARSM, Clinician-Administered Rating Scale for Mania; HAMD, Hamilton Depression Rating Scale; FGA, first-generation antipsychotic; PANSS, Positive and Negative Syndrome Scale; SAPS, Scale for the Assessment of Positive Symptoms; SANS, Scale for the Assessment of Negative Symptoms; SGA, second-generation antipsychotic; YMRS, Young Mania Rating Scale; S.D., standard deviation.

[a] Study also included patients with major depressive disorder (MDD).

[b] Only baseline data used.

[c] We used the description of symptomatic state as in the original articles.

contribute to the results. Higher HAMD scores were associated with a greater likelihood of activation in the right amygdala ($x=22$, $y=-6$, $z=-12$; $r^2=0.53$, $p=0.001$, voxels = 79) whereas higher YMRS scores were positively correlated with the likelihood of activation in the right thalamus ($x=6$, $y=-18$, $z=10$; $r^2=0.2$, $p=0.002$, voxels = 70).

Comparison of SZ to BD

Compared to SZ patients, patients with BD were more likely to activate the left pulvinar thalamus (Table 3, Fig. 2). Conversely, patients with SZ were more likely to activate the cuneus bilaterally (BA 18) (Table 3, Fig. 2). Age and sex did not contribute to differences between diagnostic groups. Differences between the two disorders in amygdala activation were negatively correlated with antipsychotic dose ($x=22$, $y=-6$, $z=-12$; $r^2=0.98$, $p=0.001$, voxels = 832).

Discussion

The results of this quantitative meta-analysis demonstrate that SZ and BD are associated with largely distinct patterns of neural engagement during facial affect processing. During facial affect processing: (*a*) patients with BD showed increased likelihood of activation within subcortical regions of the facial affect processing network (parahippocampus/amygdala, thalamus and putamen) and reduced likelihood of activation within cortical regions, specifically the ventrolateral PFC compared to healthy individuals, (*b*) patients with SZ showed reduced likelihood of activation throughout the entire facial affect processing network and increased likelihood of activation in the cuneus (and adjacent posterior cortical regions) compared to healthy individuals. Additional differences were found when the two patient groups were compared to each other. Patients with BD showed greater likelihood of activation in thalamic regions compared to patients with SZ; in the reverse comparison, SZ patients showed increased likelihood of activation in the posterior visual association cortices.

Our findings when comparing patients with either SZ or BD to healthy individuals are in line with several recent meta-analyses on this topic (Li *et al.* 2010; Chen *et al.* 2011; Sugranyes *et al.* 2011; Anticevic *et al.* 2012; Delvecchio *et al.* 2012; Taylor *et al.* 2012). Therefore, here we focus mostly on the comparison between SZ and BD so as to draw inferences about their pathophysiological boundaries. We begin by describing the functional properties of the neural network involved in facial affect processing as these are relevant to the interpretation of the differences between patients with SZ and patients with BD.

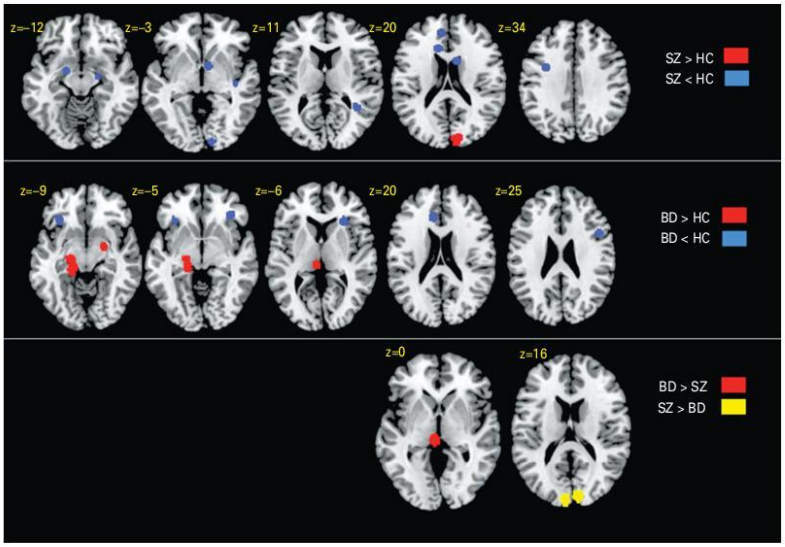

Fig. 2. Activation likelihood estimation results. Top row: patients with schizophrenia (SZ) compared to healthy controls (HC). Middle row: patients with bipolar disorder (BD) compared to HC. Bottom row: patients with SZ compared to patients with BD. $p < 0.05$ false discovery rate corrected.

Functional properties of the facial affect processing network

In fMRI studies, facial emotional expressions engage anatomically distributed cortical and subcortical regions with evidence of relative specialization (Adolphs, 2002; Haxby *et al*. 2002; Phan *et al*. 2002; Murphy *et al*. 2003; Fairhall & Ishai, 2007; Fusar-Poli *et al*. 2009; Pessoa & Adolphs, 2010; Vytal & Hammann, 2010; Dima *et al*. 2011). Within cortical regions, visual stimuli are first processed in the primary (BA 17) and extrastriate visual cortices (BA 18, 19), where they are rapidly categorized according to their physical characteristics (Lachaux *et al*. 2005). Information is then transmitted to temporal regions, notably the fusiform gyrus. The fusiform gyrus is robustly associated with encoding facial features and identity (Haxby *et al*. 2002) but is also crucially involved in facial affect processing (Pizzagalli *et al*. 1999; Vuilleumier & Pourtois, 2007), particularly in the early categorization of facial expressions (Pizzagalli *et al*. 2002; Tsuchiya *et al*. 2008). Involvement of the medial PFC is elicited by multiple tasks that require conscious attribution or judgment of mental states, dispositional traits or intentions of one's self or of others (Phillips *et al*. 2003; Amodio & Frith, 2006; Etkin *et al*. 2011). Engagement of the ventrolateral PFC relates primarily to the contextual or social appropriateness of responses to emotional cues (Berthoz *et al*. 2002; Quirk & Beer, 2006; Spreng *et al*. 2009). Among the subcortical regions, the amygdala is thought to reflect arousal (Critchley *et al*. 2005) or biological salience (Santos *et al*. 2010) associated with stimuli. The insula is engaged during observation and imitation of facial emotional expressions and is thought to contribute to the modulation of action representation (mirroring) (Carr *et al*. 2003) and agency (self or other) (Farrer *et al*. 2003) on emotional processing. Finally, higher-order thalamic nuclei also support facial affect processing. The pulvinar thalamus is directly involved in visual perception (Pessoa & Adolphs, 2010), particularly in directing and maintaining attention towards salient stimuli (Desimone *et al*. 1990). A similar role is ascribed to the medial dorsal thalamus, often referred to as the 'limbic thalamus' (Armstrong, 1990), in addition to its function in emotional responsiveness and memory retrieval (Taber *et al*. 2004).

Emotional modulation of the face processing network is reduced in SZ and increased in BD

In the contrast between emotional and neutral facial expressions, patients with SZ show reduced activation within the amygdala/parahippocampus compared to both BD patients and healthy individuals. This has

Table 3. *Activation likelihood estimation (ALE) results for facial emotion processing in schizophrenia and bipolar disorder.*

Brain region	Gyrus	BA	Laterality	Site of maximum ALE			Volume (mm^3)	Maximum ALE value
				x	y	z		
Schizophrenia > Healthy individuals								
Occipital	Cuneus	18	Right	10	−88	20	416	0.01
Schizophrenia < Healthy individuals								
Frontal	Medial frontal	9	Left	−8	46	22	360	0.01
	Precentral	6	Left	−34	2	32	856	0.01
Limbic	Parahippocampal	28	Left	−18	−2	−14	1104	0.01
	Anterior cingulated	32	Left	−12	26	22	408	0.01
	Amygdala	–	Right	20	−10	−8	384	0.01
	Insula	13	Right	40	−18	2	376	0.01
Occipitotemporal	Fusiform	37	Right	38	−48	12	592	0.01
Occipital	Inferior occipital	17	Right	12	−92	−4	328	0.01
Basal ganglia	Caudate	–	Right	8	4	−4	408	0.01
Thalamus	Medial dorsal	–	Right	10	10	18	1016	0.01
Bipolar disorder > Healthy individuals								
Limbic	Amygdala		Left	−18	−4	−14	1176	0.01
			Right	24	−4	−14	392	0.01
	Parahippocampal	28	Left	−20	−18	−8	1176	0.009
		35	Left	−18	−34	−10	248	0.009
		28	Left	−18	−28	−6	248	0.009
Thalamus	Pulvinar		Left	−6	−26	4	368	0.01
Bipolar disorder < Healthy individuals								
Frontal	Inferior frontal	47	Right	36	34	−2	2368	0.01
		47	Left	−34	28	−8	352	0.01
		44	Right	48	12	28	304	0.01
Limbic	Anterior cingulate	32	Left	−10	30	18	200	0.01
Basal ganglia	Caudate		Right	28	26	8	2368	0.01
Bipolar disorder > Schizophrenia								
Thalamus	Pulvinar		Left	−5	−26	6	336	1.9
Schizophrenia > Bipolar disorder								
Occipital	Cuneus	18	Left	−6	−92	18	1144	1.7
		18	Right	10	−88	20	416	1.7

BA, Brodmann area.
Talairach and Tournoux coordinates: x = sagittal, y = coronal, z = axial.

been attributed to abnormally elevated responses to neutral facial stimuli (Aleman & Kahn, 2005; Holt *et al*. 2006; Surguladze *et al*. 2006; Hall *et al*. 2008; Anticevic *et al*. 2012) and is thought to reflect abnormal salience attribution (Kapur, 2003). However, Gur *et al*. (2007*b*) reported that limbic activation was abnormally elevated in patients with SZ when they failed to identify facial expressions. This information, combined with behavioral evidence that SZ is associated with deficits in correct facial affect identification, suggests that patients find these stimuli ambiguous regardless of valence (Pinkham *et al*. 2007). It could therefore be argued that it is ambiguity (Santos *et al*. 2010), rather than inappropriate attribution, that drives limbic recruitment in SZ. Additionally, visual processing of facial stimuli (regardless of emotional expression) in SZ may be impaired from the early stages of sensory perception (Chen *et al*. 2009). Consistent with this observation we found reduced likelihood of recruitment in SZ within the visual and fusiform cortices involved in early stimulus categorization (Pizzagalli *et al*. 2002; Lachaux *et al*. 2005; Tsuchiya *et al*. 2008). Difficulties in stimulus categorization are also likely to increase stimulus ambiguity. It is therefore possible that a more general dysfunction in visual perception contributes to abnormal facial affect processing in SZ (Kumar *et al*. 2010; Green *et al*. 2011).

In BD, emotion seems to be associated with increased modulation of subcortical regions of the face processing network compared to healthy individuals

and to patients with SZ. BD was associated with increased engagement of the pulvinar, which indicates enhanced attention towards emotionally salient stimuli from the early stages of visual processing (Pessoa & Adolphs, 2010). Neural engagement in BD patients was also amplified in the parahippocampal gyrus/amygdala. Greater amygdala engagement in BD is congruent with the notion of increased salience of or arousal to emotional stimuli (Critchley *et al.* 2005; Santos *et al.* 2010) whereas increased parahippocampal activation may index greater contextual appraisal (Gerdes *et al.* 2010). Consistent with the primary literature, our analysis points to a positive association between level of affective symptoms and degree of signal enhancement in subcortical (Altshuler *et al.* 2005; Foland *et al.* 2008; Bermpohl *et al.* 2009) but not cortical regions (Foland-Ross *et al.* 2012).

The ACC and the ventral PFC are known to play a crucial role in evaluative and regulatory functions during emotional processing, which may map on different subdivisions of these regions (Phillips *et al.* 2003; Etkin *et al.* 2011). It has been proposed that dorsal subdivisions are primarily involved in appraisal and generation of affective states and their corresponding somatic markers (Critchley, 2005; Walton *et al.* 2011). By contrast, ventral subdivisions are thought to exert a regulatory (mostly inhibitory) influence on the amygdala and other subcortical and posterior cortical regions (Ochsner & Gross, 2005; Quirk & Beer, 2006). Therefore, the decreased engagement of the ventral ACC and PFC identified here (Table 3) is indicative of a greater impact of BD on regions relating to emotional regulation.

Increased engagement in posterior midline cortices may typify SZ but not BD

SZ was associated with increased likelihood of engagement within the cuneus compared to healthy individuals and patients with BD (Table 3). This was coupled to reduced engagement within the primary visual cortex in the inferior occipital gyrus compared to healthy individuals. The cluster of overactivation identified corresponds to the medial cuneus, which is part of the extrastriate visual cortex (Iaria *et al.* 2008) and thus involved in higher-order processing of visual information. The function most consistently ascribed to the cuneus relates to early stimulus categorization. In this respect the cuneus may enlist mnemonic and/or attentional mechanisms towards features that distinguish categories and thus modulate the quality or quantity of visual information reaching later processing stages (Sergent *et al.* 1992; Vanni *et al.* 2001). This pattern of overactivation within higher-order visual cortices in SZ was also observed by Taylor *et al.* (2012) in a meta-analysis of fMRI studies of emotional perception and by Seiferth *et al.* (2009) in a study of facial affect processing in adolescents with SZ. Taken together, these findings suggest a significant role for the visual cortices in SZ that is probably already present at the earliest stages of the illness. The nature of this overactivation within higher-order visual regions in SZ is unclear but the prevailing view is that it represents a 'compensatory' response to deficits in integrating visual information (Seiferth *et al.* 2009; Taylor *et al.* 2012). Visual abnormalities in SZ are not limited to the perception of facial expressions but have been reported in multiple paradigms including motion and form perception, spatial frequency and location discrimination, perceptual organization and backward masking (Butler *et al.* 2008; Kumar *et al.* 2010; Green *et al.* 2011).

Methodological considerations

Meta-analyses allow the synthesis of data from the primary literature but some limitations are evident. First, we accepted the results of individual studies as reported and not weighted based on the threshold of significance used in each original study. Second, although most patients were remitted when scanned (Table 2), the level of symptomatology impacted on brain activity. The effect of symptoms seems to exaggerate rather than confound diagnostic differences between the two disorders. Third, the impact of medication on our results cannot be easily evaluated. Medication regimes for SZ and BD are inherently different, with the exception of antipsychotic medication. Current evidence suggests predominantly ameliorative effects of antipsychotic medication on facial affect processing in SZ and in BD (Phillips *et al.* 2008; Cabral-Calderin *et al.* 2010). In this study antipsychotic dose seemed to reduce group differences in amygdala/parahippocampal engagement. Antipsychotic medication is known to influence the function of the amygdala (Aleman & Kahn, 2005) but the effect observed here suggests that it minimizes diagnostic differences. Fourth, a further limitation is the variability in the types of tasks used in individual source studies, which precludes separate analyses per task type because of small sets sizes. As the primary literature expands, more task-specific meta-analyses should be possible in the future. Fifth, it would be desirable to evaluate a range of study-wise factors that may influence both the nature and the topography of the results. These factors may include clinical information about age of onset or illness severity, task performance and details of data acquisition and analysis. The considerable variation in study design within the primary literature (Tables 1 and 2) unfortunately precludes

a quantitative assessment of all of these factors and we therefore focused on those for which we had sufficient information. However, our results achieved a degree of robustness that suggests that we captured the most replicable differences between patients with BD or SZ. Finally, our meta-analysis provides an estimate of the probability of differential activation in brain regions when comparing diagnostic groups and not the mean activation difference in these regions. Therefore, traditional measures of heterogeneity and publication bias that are based on the effect size of group differences are not applicable.

Conclusions

Affective dysfunction is a prominent feature of SZ and BD. This meta-analysis focused on a particular component of affective processing relating to the identification of facial affect, where we demonstrate significant diagnostic differences. We confirmed that, in SZ, affective modulation was reduced in the amygdala and additionally we show the same effect throughout the traditional face processing network. This reduction in affective modulation was previously designated to inappropriate attribution of salience to neutral stimuli (Kapur, 2003). The overactivation in higher-order visual cortices observed here supports an alternative explanation; facial affect identification deficits in SZ may arise from abnormalities in visual perception and represent another dimension of poor stimulus categorization. Our results also indicate that, in BD, there is increased affective modulation within the subcortical regions of the face processing network linked with underengagement of ventral PFC regions considered important in cognitive control. In our previous study on BD we were able to show the differential impact of positive and negatively valenced facial expressions on the face processing network (Delvecchio *et al*. 2012). The effect of valence on the neurobiology of affective facial processing in SZ remains unresolved because of the limited availability of relevant original studies but it would be worth pursuing in future research. Further work would also be required to assess the effect of diagnosis of SZ or BD on emotional expression and subjective experience of emotion, two aspects of affective processing not addressed here.

Declaration of Interest

None.

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Appendix 3: Original paper on: **"The effect of ANK3 bipolar-risk polymorphisms on the working memory circuitry differs between lociand according to risk-status for bipolar disorder." American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 2015; 9999: 1–9.**

RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART B
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The effect of *ANK3* bipolar-risk polymorphisms on the working memory circuitry differs between loci and according to risk-status for bipolar disorder

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Polymorphisms at the rs10994336 and rs9804190 loci of the Ankyrin 3 (*ANK3*) gene have been strongly associated with increased risk for bipolar disorder (BD). However, their potential pathogenetic effect on BD-relevant neural circuits remains unknown. We examined the effect of BD-risk polymorphisms at rs10994336 and rs9804190 on the working memory (WM) circuit using functional magnetic resonance imaging (fMRI) data obtained from euthymic patients with BD (n = 41), their psychiatrically healthy first-degree relatives (n = 25) and unrelated individuals without personal or family history of psychiatric disorders (n = 46) while performing the N-back task. In unrelated healthy individuals, the rs10994336-risk-allele was associated with reduced activation of the ventral visual cortical components of the WM circuit while the rs9804190-risk-allele was associated with inefficient hyperactivation of the prefrontal cortical components of the WM. In patients and their healthy relatives, risk alleles at either loci were associated with hyperactivation in the ventral anterior cingulate cortex. Additionally, Rs9804190-risk-allele carriers with BD evidenced abnormal hyperactivation within the posterior cingulate cortex. This study provides new insights on the neurogenetic correlates of allelic variation at different genome-wide supported BD-risk associated *ANK3* loci that support their involvement in BD and highlight the modulatory influence of increased background genetic risk for BD. © 2014 Wiley Periodicals, Inc.

Key words: bipolar disorder; relatives; high-risk; working memory; genetic; polymorphism; ANK3

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INTRODUCTION

Allelic variation in the Ankyrin3 (*ANK3*) gene located on chromosome 10q21.2 has been most convincingly associated with increased risk for bipolar disorder (BD). The first report concerned a genome-wide association between BD and a single nucleotide polymorphism (SNP) at rs9804190 identified in two independent samples from the US and Germany [Baum et al., 2008]. This association signal within a 70 kilobase region at the 3′ end of the gene was later confirmed in a larger study by the Psychiatric GWAS Consortium Bipolar Disorder Working Group [Sklar et al., 2011]. Three linked susceptibility loci at rs10994336 [Ferreira et al., 2008; Lett et al., 2011; Tesli et al., 2011], rs10994397 [Sklar et al., 2011] and rs1938526 [Takata et al., 2011; Lee et al., 2011; Dedman et al., 2012] have also been identified within a 250 kilobase region at the 5′ end of the gene. The association signals within the 3′ and 5′ regions do not overlap and there is no evidence of linkage disequilibrium or other interaction between the corresponding SNPs [Schulze et al., 2009]. These two regions are therefore considered as two independent genetic risk factors for BD.

The biological mechanisms linking allelic variation in the *ANK3* gene to increased risk for BD have yet to be clearly defined. The *ANK3* gene encodes for multiple protein isoforms of Ankyrin-G (AnkG) [Kordeli et al., 1995], a multi-functional protein with several distinct domains including spectrin- and trans-membrane binding domains. Brain-specific isoforms of AnkG are localized in the nodes of Ranvier and at axonal initial segments (AIS) [Kordeli et al., 1995]. AnkG is involved in maintenance of neuronal polarity [Rasband, 2010] and in the clustering of ion gated channels

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required for action potential generation and propagation [Zhou et al., 1998; Rasband, 2010]. Alterations in AnkG sequence or intracellular levels could disrupt these mechanisms and affect the function of neural circuits involved in mood and cognition. Congruent with this hypothesis, reduced *ANK3* expression of brain-specific transcripts in mouse models affects AIS throughout the brain [Leussis et al., 2013]. These mice also exhibit a number of traits considered relevant to BD, specifically increased risk taking behaviour (decreased latency in the elevated plus maze and light-dark transition), greater reward salience (decreased latency to approach food during novelty-suppressed feeding and increased sucrose preference), and increased reactivity to chronic stress (increased forced swim test immobility and elevated baseline and reactive corticosterone levels) [Leussis et al., 2013].

In human post-mortem samples, the BD-risk alleles have been associated with reduced neuronal ANK3 expression in multiple brain regions [Roussos et al., 2012; Rueckert et al., 2013]. However, in healthy individuals there are significant differences in the phenotypic traits associated with allelic variation at the 5′ compared to the 3′ *ANK3* region. Behaviorally, 5′ risk-allele carriers (rs10994336) show increased anxiety-related temperamental traits [Roussos et al., 2011] while 3′ risk-allele carriers (rs9804190) show abnormalities in psychosis-related traits [Roussos et al., 2012]. White matter connectivity is reduced in 5′ risk-allele carriers (rs10994336) but not in 3′ risk-allele carriers (rs9804190) [Linke et al., 2012]. In terms of cognitive function, 5′ risk-allele carriers (rs10994336), but not 3′ risk-allele carriers (rs9804190), underperform in tasks of sustained attention and set shifting [Ruberto et al., 2011; Hatzimanolis et al., 2012; Linke et al., 2012; Zhang et al., 2013]. The one phenotypic trait shared by risk-alleles in both 3′ and 5′*ANK3* regions is working memory disruption [Ruberto et al., 2011; Roussos et al., 2012] which is also a documented feature of BD.

Disruption in working memory (WM) circuitry in BD has been associated both with disease expression [Adler et al., 2004; Lagopoulos et al., 2007; Frangou et al., 2008; Townsend et al., 2010; Jogia et al., 2012; Pomarol-Clotet et al., 2012; Fernández-Corcuera et al., 2013] and familial risk [Drapier et al., 2008; Thermenos et al., 2010; Thermenos et al., 2011]. Disease expression is associated with diminished function in dorsolateral frontoparietal regions involved in information encoding and maintenance [Adler et al., 2004; Lagopoulos et al., 2007; Frangou et al., 2008; Townsend et al., 2010; Jogia et al., 2012; Pomarol-Clotet et al., 2012; Fernández-Corcuera et al., 2013] and with failure to deactivate the default mode network (DMN). The DMN consists of mostly midline regions exhibiting prominent decreases in activity during focused attention [Raichle et al., 2001]. Regions identified as consistent features of the DMN include the medial prefrontal cortex (MPFC), the anterior (ACC) and posterior cingulate cortex (PCC) and precuneus. In BD this failure of deactivation has been noted in the MPFC and ACC [Jogia et al., 2012; Pomarol-Clotet et al., 2012; Fernández-Corcuera et al., 2013]. The failure to suppress ACC activation during the N-back has also been reported in unaffected first-degree relatives of patients and is likely to represent a genetically mediated vulnerability trait for BD [Drapier et al., 2008; Thermenos et al., 2010].

The current study examined the effect of SNP rs10994336 and rs9804190 on the neural circuitry subserving WM in a sample of 112 individuals comprising euthymic patients with BD (n = 41), their psychiatrically healthy first-degree relatives (n = 25) and unrelated healthy individuals (n = 46). The study aimed to identify the neural mechanisms associated with the two independent risk-conferring loci for BD. We tested whether the pathogenetic effect of the risk alleles at rs10994336 and rs9804190 independently contribute to failure to suppress DMC activation during the n-back task in patients and their healthy relatives, and whether a similar effect would be observed in unrelated individuals without a personal or family history of psychiatric disorders.

SUBJECTS AND METHODS

Participants

All participants were selected from the VIBES study cohort which comprises 75 families identified through a proband with BD type I and screened to exclude pedigrees with schizophrenia or schizophrenia spectrum disorders. Details of the VIBES rationale and design have been reported previously [Frangou, 2009]. The sample considered in the present study comprised 41 euthymic patients with BD, 25 of their psychiatrically healthy first-degree relatives and 46 healthy unrelated individuals, all of white British ancestry (Table I). The study received institutional ethical approval. All individuals provided written informed consent prior to participation.

All participants were assessed by trained psychiatrists with patient or non-patient versions of the Structured Clinical Interview for Interview (SCID) [First et al., 2002a,b], the Hamilton Depression Rating Scale (HDRS) [Hamilton, 1960], the Young Mania Rating Scale (YMRS) [Young et al., 1978], the expanded Brief Psychiatric Rating Scale (BPRS) [Lukoff et al., 1986] and the Wechsler Adult Intelligence Scale-Revised (WAIS-R) [Wechsler, 1981]. Patients fulfilled criteria for BD type I based on the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, revised [American Psychiatric Association, 1994]. We included only psychiatrically healthy relatives of BD probands based on the absence of a personal lifetime history of any psychiatric disorder. Unrelated healthy individuals without a personal or family history of psychiatric disorders were selected to match patients and relatives on age, sex and IQ. Exclusion criteria for all participants were current and hereditary neurological disorders, DSM-IV lifetime drug or alcohol dependence or drug or alcohol abuse in the preceding 6 months and contraindications to MR imaging. Prior to cognitive and MRI evaluation, patients were required to have been in remission, defined as scoring below 7 in HDRS and YMRS, for a minimum of 1 month based on prospective weekly assessments, and to have remained on the same medication type and dose for at least 6 months. There was a significant effect of group on all symptom rating scales ($F_{(2,112)} > 9.82$, $P < 0.001$) with patients having higher scores than healthy relatives and unrelated healthy individuals; there was no difference between the latter two groups (Table I). The HDRS, YMRS and BPRS were highly correlated with each other (all $r = 0.73$, $P < 0.0001$). As only the BPRS is suited for non-patient populations (healthy relatives and unrelated healthy individuals) this scale was chosen to control for psychopathology in subsequent analyses.

Thirty BD patients were on psychotropic medication; 12 on antipsychotics (7 on atypical, 2 on typical and 3 on both), 21 on

TABLE I. Study Sample

	Unrelated healthy individuals N = 46	Patients with BD N = 41	Healthy relatives N = 25
Demographic variables			
Age (years)	40.3 (13.2)	44.3 (11.9)	39.7 (13.7)
Sex (male/female)	25/21	20/21	13/12
Clinical features			
HDRS total score[a]	0.1 (0.5)	4.8 (5.3)	0.14 (0.4)
YMRS total score[a]	0.2 (0.6)	1.4 (3.0)	0.0 (0.0)
BPRS total score[a]	24.3 (0.7)	27.5 (4.0)	24.1 (0.4)
Age of onset (years)	n/a	24.7 (8.0)	n/a
Duration of illness (years)	n/a	20.2 (10.5)	n/a
Depressive episodes (n)	n/a	5.7 (7.5)	n/a
Manic episodes (n)	n/a	5.6 (7.7)	n/a
Cognitive task performance			
IQ	112.6 (14.5)	117.9 (17.9)	115.8 (18.5)
1-Back accuracy (% correct)	100	100	100
1-Back response time (sec)	0.6 (0.3)	0.5 (0.2)	0.5 (0.2)
3-Back accuracy (% correct)[b]	72.1 (17.2)	68.9 (19.7)	90.1 (15.4)
3-Back response time (sec)	0.8 (0.4)	0.8 (0.3)	0.7 (0.2)

Except for sex, all data are presented as mean (standard deviation); BD, bipolar disorder; BPRS, Brief Psychiatric Rating Scale; HDRS, Hamilton Depression Rating Scale; YMRS, Young Mania Rating Scale
[a]Patients > controls and relatives ($P < 0.001$).
[b]Relatives > controls and patients ($P = 0.003$).

mood stabilisers (lithium = 15, sodium valproate = 6), and 13 on selective serotonin reuptake inhibitors. None received anticholinergics or benzodiazepines. Medicated and unmedicated BD patients did not differ in age of onset, illness duration, IQ, HDRS, YMRS and BPRS total scores (all $P > 0.31$).

DNA Extraction and Genotyping

DNA was obtained from buccal swabs using conventional procedures. The *ANK3* rs10994336 (risk allele T) as well as the *ANK3* rs9804190 (risk allele C) genotype were determined by the TaqMan allelic discrimination assay (Applied Biosystems, Assay ID C_31344821_10). Endpoint analysis was performed using the Applied Biosystems 7900HT Fast Real-Time PCR System. Genotypes were called with the SDS 2.3 software (Applied Biosystems) and the output was checked visually to ensure genotypes fell into distinct clusters. Call rate was 100% as buccal swabs were repeated for seven individuals for whom initial genotyping was undetermined. Accuracy was assessed by duplicating 15% of the sample. Reproducibility was 100%.

Within each group (patients, healthy relatives, unrelated healthy individuals) homozygote and heterozygote risk-allele carriers for each SNP were considered as detailed in Supplemental Tables S1 and S2. There was no effect of genotype or group-by-genotype interaction on age or sex (Tables S1 and S2).

Neuroimaging

Experimental paradigm. The n-back task was employed in a block design incorporating alternating experimental and sensorimotor control conditions. A series of letters in yellow font were displayed on a blue screen for 2 sec each. Participants were instructed to indicate by a button press whether the letter currently displayed matched the letter from the preceding n trials. In the sensorimotor control (0-back) condition, the letter "X" was the designated target. In the experimental conditions (1, 2, 3-back) the target letter was defined as any letter that was identical to the one presented in the preceding one, two or three trials. There were 18 epochs in all, each lasting 30 sec, comprising 14 letters with a ratio of target to non-target letters ranging from 2:12 to 4:10 per epoch. The entire experiment lasted 9 min and included a total of 49 target and 203 non-target stimuli. To avoid any systematic order effects the conditions were pseudo-randomised. Performance was evaluated in terms of reaction time to target letters and accuracy (% correct responses). Group differences in accuracy were examined using analysis of variance followed by pairwise comparisons with Bonferroni correction.

Acquisition parameters. Gradient echo planar magnetic resonance (MR) images were acquired using a 1.5-Tesla GE Neuro-optimized Signa MR system (General Electric, Milwaukee, WI, USA) fitted with 40 mT/m highspeed gradients, at the Maudsley Hospital, London. Foam padding and a forehead strap were used to limit head motion. A quadrature birdcage head coil was used for radio frequency (RF) transmission and reception. A total of 180 T2*-weighted MR brain volumes depicting blood-oxygenation level-dependent (BOLD) contrast were acquired at each of 36 near-axial planes parallel to the inter-commissural (AC-PC) plane; repetition time (TR) = 3000 ms, echo time (TE) = 40ms, slice thickness = 3mm, voxel dimensions = 3.75 × 3.75 × 3.30mm, interslice gap = 0.3 mm, matrix size = 64 * 64, flip angle = 90°. Prior to each acquisition sequence, four dummy data acquisition scans were performed to allow the scanner to reach a steady state in T1 contrast. During the same session, a high-resolution T1-weighted structural image was acquired in the axial plane for subsequent co-registration (inversion

recovery prepared, spoiled gradient-echo sequence; TR = 18ms, TE = 5.1 ms, TI = 450 ms, slice thickness = 1.5 mm, voxel dimensions = 0.9375 × 0.9375 × 1.5 mm, matrix size 256 * 192, field of view = 240 × 180 mm, flip angle = 20°, number of excitations = 1.

Neuroimaging data analysis. All analyses were implemented using Statistical Parametric Mapping (SPM8) (www.fil.ion.ucl.ac.uk/spm/software/spm8/). The BOLD images were realigned to the fifth volume and corrected for interscan movements by means of a rigid body transformation with three rotation and three translation parameters. Subsequently, the 180 fMRI images were spatially normalized to the standard template of the Montreal Neurological Institute (MNI) and re-sampled to a voxel size of 2 × 2 × 2mm. Finally, the images were smoothed using an 8 mm full-width-half-maximum Gaussian kernel.

The smoothed single-subject images were analyzed via multiple regression using a standard linear convolution model, with vectors of onset representing the 1, 2, 3-back and the 0-back condition as the sensorimotor control. Serial correlations were removed using an AR(1) model. A high pass filter (128s) was applied to remove low-frequency noise.

As the effect of any single SNP on neural networks is expected to be subtle, all subsequent analyses were restricted to the 3-back condition because (a) individual differences in cognitive and neural efficiency are more apparent at high WM load [Gevins and Smith, 2000], and (b) the effect of diagnosis in patients with BD and their relatives is also most consistently seen at high WM load [Jogia et al., 2012; Palaniyappan and Liddle, 2014]. Images representing the 3-back versus 0-back contrast from each subject were entered in second level random-effects.

First, we investigated the main effect of each risk-SNP (rs10994336 and rs9804190) and their interaction on the WM circuitry in healthy unrelated individuals. This analysis allowed us to relate our findings to the literature that has examined the effect of *ANK3* only in unrelated healthy individuals. Second, full factorial ANCOVA was used to the effect of each SNP and their interactions in patients, healthy relatives and unrelated healthy individuals with BPRS and accuracy as covariates. Suprathreshold clusters were identified using voxel-level Family Wise Error (FWE) correction of $P < 0.05$. Stereotactic coordinates of the peak maxima of the suprathreshold clusters were converted (www.mrc-cbu.cam.ac.uk/Imaging/mnispace.html) from the Montreal Neurological Institute spatial array (www.mni.mcgill.ca) to that of Talairach and Tournoux [Talairach and Tournoux, 1988]. Mean signal change from suprathreshold clusters was extracted using the MarsBaR toolbox (http://marsbar.sourceforge.net/) and was entered in correlation analyses to examine the effect of age of onset, duration of illness, number of episodes, and medication dose at the time of scanning (lithium and antipsychotic). Threshold for statistical significance was set at $P < 0.005$ following Bonferroni correction.

RESULTS

Effect of ANK3 Allelic Variation on Clinical Features

Rs10994336 or rs9804190 risk associated patients had significantly higher HDRS, YMRS and BPRS scores compared to all other groups ($F_{(2,112)} > 6.5$, $P < 0.02$) (Supplemental Tables S1 and S2). There was no effect of genotype on patients' age of onset, duration of illness and number of mood episodes ($t_{39} < 1.2$, $P > 0.2$).

Effect of ANK3 Allelic Variation on Cognitive Task Performance

There was no effect of group, genotype or group x genotype interaction for either SNP on general intellectual ability or response time ($P > 0.1$) (Table I, Supplemental Tables S1 and S2). In contrast, there was a significant effect of group on accuracy for the 3-back condition only, where relatives were significantly better than both other groups ($F_{(2,112)} > 24.31$, $P < 0.003$) (Table I). Within the relatives group, non-risk associated relatives for either rs10994336 or rs9804190 had significantly higher accuracy ($P < 0.01$) (Supplemental Tables S1 and S2).

Effect of ANK3 Allelic Variation on WM-Related Activation in Unrelated Healthy Individuals

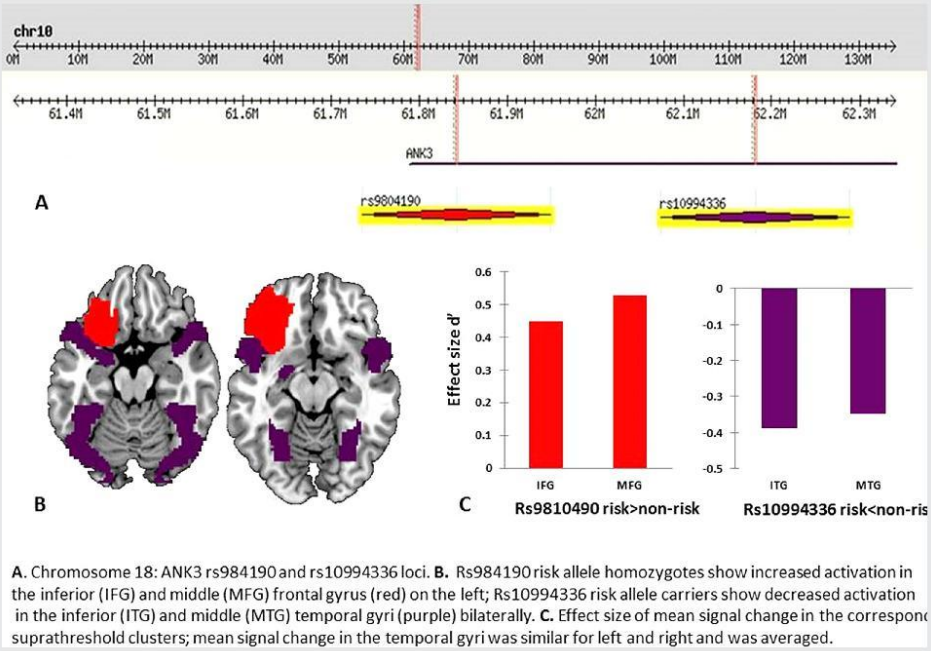
Healthy carriers of the rs10994336 risk-allele showed significantly decreased lateral temporal cortical activation within the middle (BA21) and inferior (BA20) temporal gyrus (Fig. 1). In contrast, healthy homozygotes of the rs9804190 risk-allele showed increased activation in the lateral prefrontal cortex within the inferior (BA47/11) and middle (BA46) frontal gyrus (Fig. 1). The coordinates of the peak height voxel of the corresponding suprathreshold clusters are presented in Table II.

Effect of ANK3 Allelic Variation on WM-Related Activation in BD Patients and Their Healthy Relatives

An effect of group (patients, healthy relatives, healthy unrelated individuals) was observed in the middle (BA 9 and 10) frontal gyri, in the superior and middle temporal gyri (BA 21/22) and in the ventral ACC (BA 24/32). When compared to unrelated healthy individuals, brain activation in patients was significantly (a) reduced in the left (BA9) and right middle frontal gyri (BA10) and, (b) increased in the superior and middle temporal gyri (BA21/22) on the right and in the ACC bilaterally (BA24/32). In comparison to patients, healthy relatives had greater activation in the middle frontal gyrus bilaterally. No differences were observed between unrelated healthy individuals and healthy relatives. The coordinates of the peak activations of the suprathreshold clusters are shown in Supplemental Table S3.

In patients, there were no significant correlations between mean signal change in suprathreshold clusters and age of onset, duration of illness, mood episodes or medication dose ($P > 0.1$).

For the *ANK3 rs10994336*, we found a significant genotype x group interaction in the right ventral ACC (x = 4, y = 19, z = −3, cluster size = 35, z-value = 3.67) and left ventral posterior cingulate cortex (PCC; x = −28, y = −64, z = 16, cluster size = 166, z-value = 4.10). In the right ACC, the risk T-allele was associated with increased activation in BD patients and their healthy relatives compared to unrelated healthy individuals. In the left PCC, the



A. Chromosome 18: ANK3 rs984190 and rs10994336 loci. **B.** Rs984190 risk allele homozygotes show increased activation in the inferior (IFG) and middle (MFG) frontal gyrus (red) on the left; Rs10994336 risk allele carriers show decreased activation in the inferior (ITG) and middle (MTG) temporal gyri (purple) bilaterally. **C.** Effect size of mean signal change in the correspon suprathreshold clusters; mean signal change in the temporal gyri was similar for left and right and was averaged.

FIG. 1. Effect of genotype on regional brain activation in the 3-back versus 0-back contrast in unrelated healthy individuals.

risk T-allele was related with increased activation in BD patients compared to their healthy relatives and to unrelated healthy individuals (Fig. 2).

For the *ANK3 rs9804190*, a significant genotype x group interaction was found in the right ACC (x = 4, y = 17, z = −4; cluster size= 58; z-value = 3.95) in which patients and healthy relatives who were risk C-allele homozygotes showed increased activation compared to unrelated healthy individuals (Fig. 2).

DISCUSSION

There are two key findings from this study. First, in healthy individuals without personal or family history of psychiatric disorders, the rs10994336 and rs9804190 BD-risk alleles had different effects on the working memory (WM) network, although neither affected task performance. Second, both BD-risk alleles were associated with failure to deactivate the default mode network (DMN) in patients and in their healthy relatives; accuracy was reduced in risk-associated healthy relatives.

Unrelated healthy carriers of the rs10994336 risk allele showed reduced engagement of the ventral visual cortex within the middle and inferior temporal gyri (Table II). This accords with previous reports from two independent samples which found that the largest effect size of the rs10994336 risk allele was on reduced sensitivity in target detection and increased errors of commission during the degraded symbol continuous performance task [Ruberto et al., 2011; Hatzimanolis et al., 2012]. Although the ventral visual cortex is an integral part of the WM circuitry, the core WM network involves the frontoparietal cortices [Owen et al., 2005; Leech et al., 2011; Rottschy et al., 2012]. These regions are also core components of the superordinate cognitive control network that supports a broad range of executive function tasks [Niendam et al., 2012]. Unrelated healthy rs9804190-risk allele homozygotes evidenced greater activation within the prefrontal components of the WM network although their task performance was comparable to that of the non-risk associated unrelated individuals (Table II). This pattern is typically interpreted as evidence of cortical inefficiency, and is consistent with behavioural data from an independent sample that also found that healthy rs9804190-risk-allele homozygotes underperform in a wide array of executive function tasks [Roussos et al., 2012].

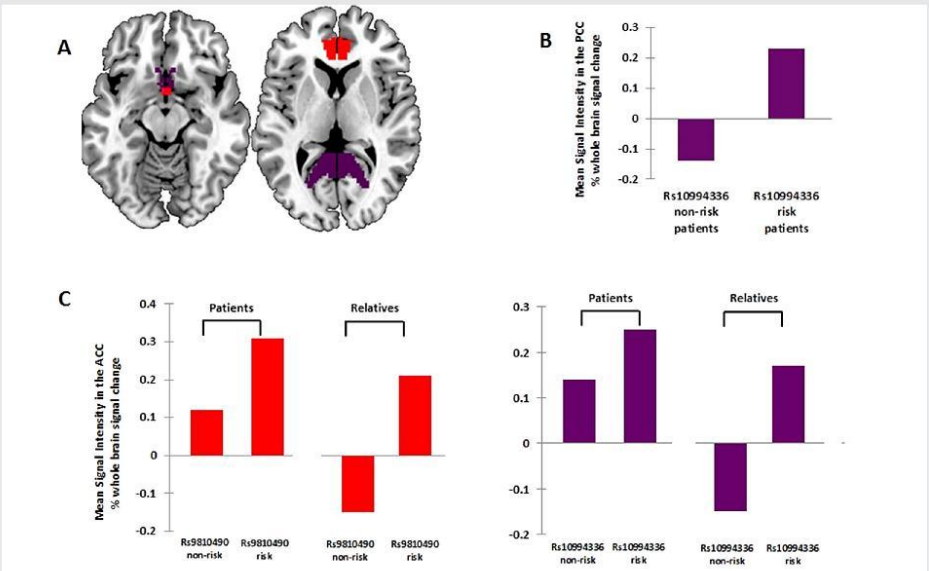
These findings suggest that in the absence of increased background genetic risk for BD or other psychiatric disorders the two *ANK3* BD-risk loci affect different regions of the WM circuitry. The reason for these regional differences is unclear. Available data suggest that 3′ risk-alleles (rs9804190) are associated with reduced transcript

TABLE II. Brain Regions Showing Significant Effects of Allelic Variation at 10994336 and rs9810490 in the 3-back versus 0-back Contrast in Unrelated Healthy Individuals

				Talairach and Tournoux coordinates				
Region	**Gyrus**	**Laterality**	**Brodmann area**	**x**	**y**	**z**	**Cluster size**	**z-value**
ANK3 rs10994336: risk allele homozygotes < non-risk allele carriers								
Temporal	Middle temporal	Left	21	−40	12	−28	120	3.41
		Right		48	−5	−15	90	3.76
				59	−14	−4	38	3.60
	Inferior temporal	Left	20	−42	−12	−24	56	3.43
		Right		44	−11	−20	90	3.80
ANK3 rs9810490: risk allele carriers > non-risk allele homozygotes								
Frontal	Middle frontal	Left	46	−40	38	20	46	3.51
	Inferior frontal	Left	47/10	−46	48	−4	51	3.48

Suprathreshold clusters significant $P < 0.05$ Family wise correction; x = sagittal; y = coronal; z = axial.

levels of brain-specific AnkG isoforms. To date, the region most commonly implicated is the cerebellum where *ANK3* expression is generally highest [Rueckert et al., 2013]. Information about other brain regions is incomplete because the available post-mortem studies have limited statistical power due to the small number of donors and incomplete brain coverage [Roussos et al., 2012; Rueckert et'al., 2013]. With regard to rs10994336, the effect of the risk allele on *ANK3* expression in the brain is unknown. The



A. Rs984190 (red) and Rs10994336 (purple) risk associated patients and relatives show increased activation in overlapping regions of the ventral anterior cingulate cortex (ACC); Additionally Rs10994336-risk associated patients show increased activation in posterior cingulate cortex (PCC). **B.** Mean signal change in the PCC seen in patients only. **C.** Mean signal change in the ACC in patients and relatives.

FIG. 2. Effect of genotype on regional brain activation in the 3-back versus 0-back contrast in patients with bipolar disorder and their psychiatrically healthy first-degree relatives.

rs10994336 polymorphism is located in an intronic region [Tesli et al., 2011] but could affect gene expression through cis- or trans-regulatory mechanisms [Quinn et al., 2010]. Alternatively, rs10994336 may be in strong linkage disequilibrium with other, yet unidentified, genetic loci that drive the effects observed here.

Rs10994336 or rs9804190 risk-associated patients and relatives showed hyperactivity within the ventral ACC. The ventral ACC is integral to a network of brain regions involved in affect processing and generation [Critchley et al., 2003] and a key component of the anterior DMN [Raichle et al., 2001; Buckner et al., 2008]. Activation within the ventral ACC is increased during the processing of arousing stimuli or during mental stress [Critchley et al., 2003]. The n-back task is quite challenging and may engender mild mental stress but it is not expected to result in ventral ACC hyperactivation. In fact, deactivation of the ventral ACC is normally observed during the n-back task within the context of anticorrelated activity between the DMN and the frontoparietal cognitive control network [Esposito et al., 2006; Leech et al., 2011].

Accordingly, healthy unrelated individuals in this study showed deactivation of the ventral ACC during the n-back task regardless of genotype. As expected, hyperactivation within the ventral ACC was observed in the patients regardless of genotype [Jogia et al., 2012; Pomarol-Clotet et al., 2012; Fernández-Corcuera et al., 2013] but it was more pronounced in rs10994336 or rs9804190 risk associated individuals. Among the healthy relatives, ventral ACC hyperactivity was only present in risk associated individuals for either risk allele. Additionally, BD carriers of the rs10994336 risk allele also showed hyperactivity within the PCC, centred on the ventral and extending to dorsal regions [Vogt et al., 2006]. The PCC has dense anatomical connections with multiple cortical and subcortical regions [Hagmann et al., 2008] and is a core component of the posterior DMN [Raichle et al., 2001; Buckner et al., 2008]. Healthy individuals performing the n-back task show deactivation in both dorsal and ventral PCC [Esposito et al., 2006; Leech et al., 2011] so the persistent PCC activation seen in patients suggests that the rs10994336 risk allele compromises the ability to deactivate this brain region.

Taken together, these findings suggest that aberrant hyperactivation within the ventral ACC is a key mechanism underlying the risk-conferring effects of rs10994336 and rs9804190 in connection to the WM circuitry. It is noteworthy that this effect appeared to require the concomitant presence of additional risk factors for BD as it was not observed in unrelated individuals who had no personal or family history of such risk factors. This is consistent with the multifactorial pathogenetic model of BD that involves interaction between multiple genetic and non-genetic risk factors [Sullivan et al., 2012]. The effect of any individual factor depends on the relative prevalence of other risk factors that are part of the same pathogenetic process. This observation is not unique to *ANK3*. Consistent with the findings reported here, several neuroimaging studies have shown differential effects of various susceptibility polymorphisms (e.g., *DISC1*, *NRG1*, *COMT*) on brain structure and function in patients, high-risk groups and unrelated healthy individuals [Addington et al., 2007; Mechelli et al., 2008; Prata et al., 2008; Narr et al., 2009; Whalley et al., 2012; Tsuchimine et al., 2013].

In conclusion, our results point to a differential effect of BD-risk associated polymorphisms at ANK3 rs10994336 and rs9804190 modulated by risk-status for the disorder. This suggests that the BD-risk conferring mechanisms associated with these genetic variants are influenced by other genetic and possibly non-genetic factors that contribute to risk status. Inability to suppress key nodes of the DMN emerged as a common final pathway through which either risk allele may contribute to the pathogenesis of BD. Mood stabilizing medications such as Lamotrigine interact with the ANK3 system through ion channels bound by AnkG to the axonal initial segment. Our results therefore lend further support to our previous study on patients with BD treated with Lamotrigine that showed "normalization" of the WM circuitry [Lang et al., 1993; Haldane et al., 2008] and suggest that ANK3-related molecular pathways may be a fruitful ground for the identification of new drug targets for BD.

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